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Differentiation of dopamine receptor types in the central nervous system of the rat

Ihor Krewsun
University of the Pacific

DIFFERENTIATION OF DOPAMINE RECEPTOR TYPES IN THE CENTRAL NERVOUS SYSTEM OF THE RAT

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES
(continued)

LIST OF FIGURES

LIST OF FIGURES
(continued)

LIST OF FIGURES
(continued)

LIST OF FIGURES
(continued)

LIST OF FIGURES
(continued)

LIST OF PLATES

INTRODUCTION

There is considerable evidence to suggest that dopamine (DA), in addition to its role as a precursor of norepinephrine (NE) and ephinephrine, has important physiological actions in its own right. One physiological action of DA seems to be that of a neurotransmitter in the mammalian brain (Hornykiewicz, 1966). In addition, there is evidence that abnormalities of dopaminergic transmission in the central nervous system (CNS) may be of clinical importance. For example, dopaminergic over activity in the mesolimbic forebrain may be a primary feature in the etiology of schizophrenia (Meltzer and Stahl, 1976).

The drugs used to treat schizophrenia act as DA antagonists in the brain (Snyder et al., 1974; Robinson et al., 1979). Drugs such as phenothiazines and butyrophenones have been shown in clinical studies to be effective in treating the fundamental symptoms of psychosis (Snyder et al., 1974). The results of animal experiments indicate that their principal mode of action is blockade of DA receptor sites in the CNS (Van Rossum, 1966). However, these neuroleptics are generally nonspecific in their effects upon DA neurons and thus, cause major undesireable side effects.

If new drugs could be discovered that were more structurally selective for different DA systems, then, perhaps these undesireable side effects could be eliminated. In order to develop such drugs, a closer look would have to be made at different DA systems in an attempt to demonstrate DA receptors which are topographically distinct and can thus be selectively regulated by both agonistic and antagonistic agents. The demonstration of more than one DA receptor in mammalian CNS is the subject of the research presented in this thesis.

LITERATURE SURVEY

I. Dopaminergic Systems in the Central Nervous System

During the past twenty years there has been an increasing realization of the importance of DA as a neurotransmitter in the mammalian brain. As a result, research has been focused on demonstrating and mapping out distinctive dopaminergic neuronal tracts in the CNS. DA is not present uniformly throughout the brain, but is localized in a number of discrete pathways that are still being identified and characterized in the brains of laboratory animals as well as in postmortem human brains. There is good evidence from histofluorescence and biochemical studies for at least six distinct DA neuronal tracts: 1) retinal DA fibers, 2) preoptic anterior hypothalamic DA neurons, 3) nigrostriatal DA tract, 4) tubero-infundibular DA tract, 5) mesolimbic DA tract, and 6) mesocortical DA neurons (Ungerstedt, 1971a). A schematic presentation of their location and connections is given in Figure 1.

The largest and by far most thoroughly investigated DA tract is the nigrostriatal system (Anden *et al.*, 1964). The cell bodies for this tract are located in the pars compacta of the substantia nigra, a pigmented nucleus in the brainstem. These cells give rise to a large pathway

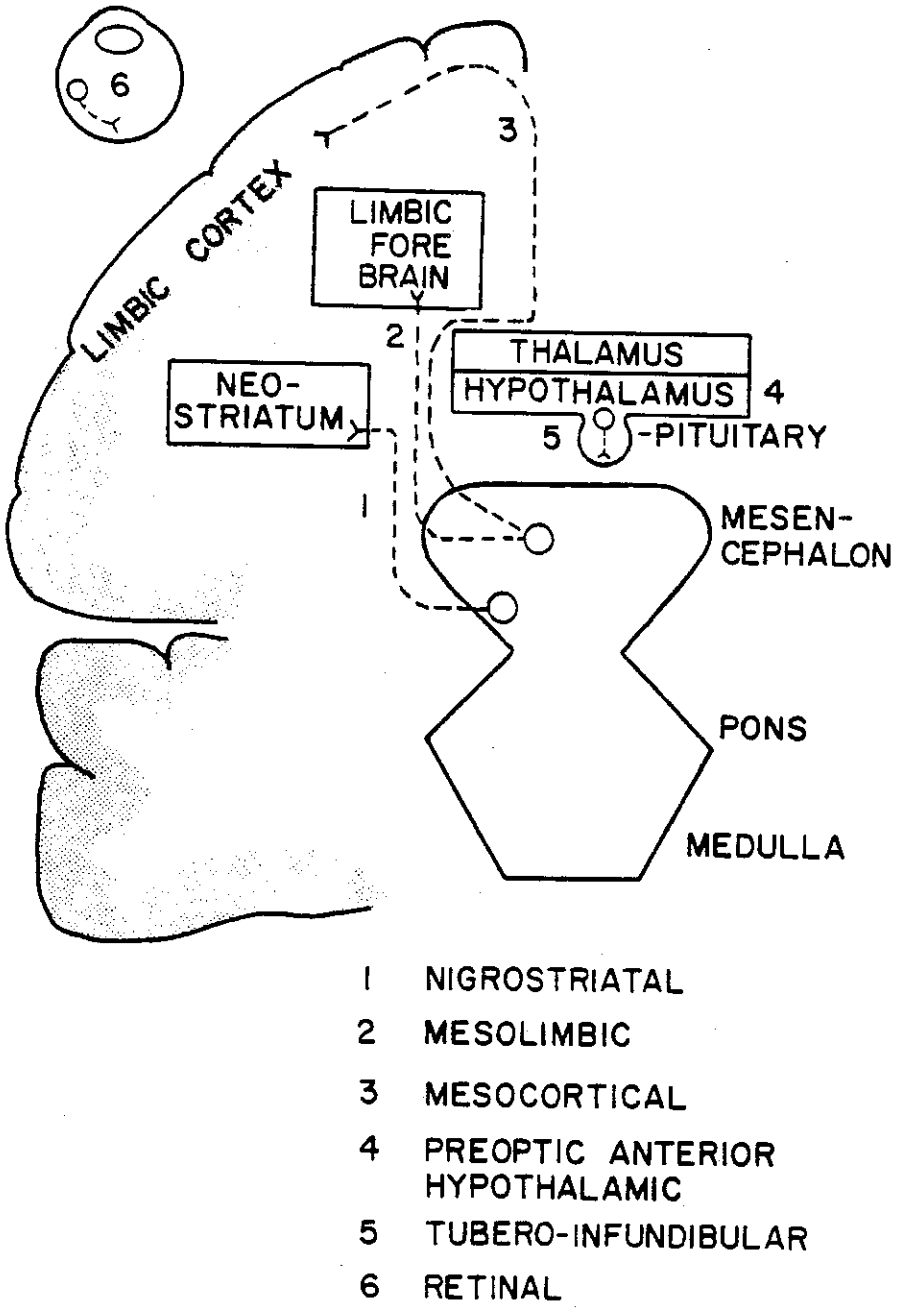


Figure 1. Major Dopaminergic Pathways (modified from Meltzer and Stahl, 1976).

that ascends through the lateral hypothalamus, enters the crus cerebri in mid-hypothalamus to mix with the myelinated bundles of the internal capsule, and then fans out in the globus pallidus, finally terminating in the caudate-putamen of the neostriatum (Ungerstedt, 1971a). The major function of the nigrostriatal DA tract is to regulate the neostriatum in its control of certain motor movements. Degeneration of nigral DA neurons is believed to be the cause of Parkinson's disease (Hornykiewicz, 1975). Blockade of DA receptors in the neostriatum with neuroleptic drugs is thought to be the cause of extrapyramidal syndrome (EPS) (Hornykiewicz, 1975).

The mesolimbic DA neuronal cell bodies are located dorsal to the interpenduncular nucleus in the ventral tegmental area and end in terminals just anterior to the caudate nucleus in the nucleus accumbens septi, the bed nucleus of the stria terminalis, and the deep portion of the olfactory tubercle (Ungerstedt, 1971). The mesolimbic DA pathway (Anden et al., 1966; Hokfelt et al., 1974) is involved in behavioral regulation; according to one popular hypothesis, schizophrenia is attributed to overactivity of this particular system (Meltzer and Stahl, 1976).

The presence of cortical DA fibers was first demonstrated by Thierry et al. (1973). Hokfelt et al. (1974) was the first to characterize the histochemical distribution of dopaminergic terminals in rat limbic cortex. They described terminal plexi in gyrus cinguli, etorhinal cortex, hippocampus, amygdaloid cortex, the most basal layers of

dorsal frontal cortex, and to a minor extent, the prepyriform cortex. It is tempting to assume that the disturbances of thinking processes that are an essential feature of many, if not all, patients in the schizophrenia spectrum are based on dysfunction of these dopaminergic neurons with cerebral cortical terminals (Meltzer and Stahl, 1976; Hokfelt et al., 1974).

Cox and Lee (1979) provide evidence for the presence of a dopaminergic system within the preoptic anterior hypothalamus. It appears that DA receptors present in this brain region mediate a fall in core temperature in the rat (Cox and Lee, 1977b) and in the cat (Quock and Gale, 1974).

The tubero-infundibular DA tract consists of dopaminergic neurons that have their cell bodies in the arcuate nucleus of the hypothalamus and have their terminals in the external layer of the median eminence. One function of the tubero-infundibular dopaminergic neurons is to exert a tonic inhibitory effect on the cells of the anterior pituitary which synthesize and release prolactin (Hokfelt and Fuxe, 1972).

In addition, there exists a small number of DA-containing neurons in the eye, which seems to send processes to both the outer and inner plexiform layers of the retina (Ehringer and Falck, 1969). The function of these retinal DA neurons has not yet been ascertained.

II. Dopaminergic Involvement in the Control of Locomotor Activity in Rats

Previous experiments suggest that DA may play a role in the control of locomotor activity in rats. It was reported by Maj et al. (1971) that rats showed an increase in locomotor activity following the systemic administration of L-dopa and a dopa decarboxylase inhibitor. An increase in locomotor activity was also induced by the systemic administration of amphetamine (Maj et al., 1972a). In rats pretreated with pimozide (DA antagonist) or phenoxybenzamine (alpha-adrenergic blocking agent), L-dopa-stimulation and that induced by amphetamine was antagonized by pimozide, while phenoxybenzamine produced complete or partial inhibition of hyperactivity induced by L-dopa or amphetamine (Maj et al., 1972a). These results show that in the stimulation of locomotor activity by these compounds, both central catecholamines, DA and NE, play a role.

Intracerebral injection of DA or drugs interfering with DA neurotransmission indicate that locomotion may be related to mesolimbic DA neurotransmission. It was found that bilateral injection of DA into the nucleus accumbens of nialamide-pretreated rats resulted in an enhancement of locomotor activity (Pijnenburg and Van Rossum, 1973). This is in agreement with Costall and Naylor (1975) who reported a dose-dependent increase in locomotor activity in rats following the bilateral injection of DA into the nucleus accumbens. Only a periodic

hyperactivity developed after similar injections into the tuberculum olfactorium and no change in behavior was observed following injections into the nucleus amygdaloideus centralis (Costall and Naylor, 1975). Pijnenburg and Van Rossum (1973) reported only a slight to moderate increase of locomotor activity following administration of DA into the caudate nucleus.

When NE was administered into the nucleus accumbens of rats, an increase in locomotor activity was noted, although less intense than after DA (Pijnenburg and Van Rossum, 1973). Later studies by Pijnenburg et al. (1975) were performed in an effort to determine whether the NE-induced increase in locomotor activity is a result of an action of NE receptors or by stimulation of DA receptors. The authors found that the locomotor stimulation induced by administration of 1 microgram of DA directly into the nucleus accumbens was completely blocked by haloperidol (HAL). The same dose of HAL also completely inhibited the effect of 5 micrograms of NE. HAL is considered as a rather specific DA antagonist both peripherally and centrally (Van Rossum, 1966), with only relatively small effects on NE receptors (Anden et al., 1970). Since a rather low dose was used, the effect of HAL upon the DA and NE-induced locomotor stimulation may be ascribed to blockade of DA receptors. Pijnenburg et al. (1975) were unable to successfully inhibit the NE-induced hypermotility with phentolamine (alpha-adrenergic antagonist) or propranolol

(beta-adrenergic antagonist), suggesting that the NE-induced stimulation of locomotor activity is indirectly associated with DA mechanisms.

Costall and Naylor (1976) found that systemic administration of DA receptor blockers was able to antagonize the increase in locomotor activity produced by bilateral application of DA to the nucleus accumbens of nialamide-pretreated rats. The neuroleptics used in these studies included the so-called classical DA receptor blockers (HAL, fluphenazine, pimozide, clothiapine), exhibiting a high degree of EPS, and atypical DA receptor blockers (clozapine, sulpiride, thioridazine (TRZ)), exhibiting a low incidence of EPS. These results further support the involvement of dopaminergic mechanisms in the control of locomotor activity.

There is good evidence to suggest that apomorphine (APO) stimulates DA receptors in rat brain (Di Chiara and Gessa, 1978; Anden et al., 1967; Iversen, 1975). APO was found to produce hypermotility in rats following various subcutaneous doses (Buus Lassen, 1974, 1977). It was also found that pretreatment of these rats with the neuroleptics chlorpromazine (CPZ), TRZ, perphenazine, clozapine, pimozide and HAL antagonized the hypermotility. These results are in agreement with Ljungberg and Ungerstedt (1978), who found that the systemic administration of HAL, TRZ, CPZ, clozapine and sulpiride to rats effectively antagonized the locomotor activity produced by APO (5.0 mg/kg, s.c.).

There is evidence to suggest that the mechanism by which APO increases locomotor activity is via the direct stimulation of the DA receptor in the nucleus accumbens (Kelly et al., 1975; Iversen et al., 1975). Other factors may also be involved. The ability of phenoxybenzamine to attenuate APO-induced hypermotility in rats suggests that an indirect stimulation of the noradrenergic system plays a role in APO-induced hypermotility (Maj et al., 1972b).

III. Dopaminergic Involvement in Apomorphine-Induced Stereotyped Behavior

Following the administration of APO, a profound change in the behavioral pattern of the rat occurs. The characteristic feature of the pattern that emerges is the compulsive repetition of senseless movements. The discontinuous sniffing initially observed in this behavioral syndrome is progressively replaced by continuous sniffing, exploratory activity and repetitive head and limb movements. When the full-blown apomorphine syndrome is reached, the rat restricts its locomotor activity to a small area of the cage while continuously gnawing, biting and licking. This drug-induced syndrome, characterized by compulsive behavior of a continuous and apparently aimless nature, is known as "stereotyped behavior" (Di Chiara and Gessa, 1978; Randrup and Munkvad, 1974).

Support for the striatum as the central site of APO stereotypy was demonstrated very early (Amsler, 1923). It

was found that the ability of APO to produce stereotyped behavior was abolished following striatal ablation. In support of this view, Ernst and Smelik (1966) showed that when administered in microquantities directly into the dorsal part of the caudate nucleus or in the globus pallidum, APO and dopa could induce stereotyped behavior. When implanted in the ventral part of the caudate nucleus, in subthalamic structures or in the substantia nigra, these agents were unable to induce stereotypy.

Since the substantia nigra appears to send fibers to the corpus striatum, the finding that this part of the mesencephalon contains DA is of great interest. In normal rats, DA-storing nerve terminals are found in the neostriatum (the caudate nucleus and putamen) (Bertler and Rosengren, 1959) and DA-containing nerve cells in the substantia nigra, mainly in the pars compacta (Kebabian and Saavedra, 1976). After electrolytic lesions in the substantia nigra or the internal capsule, the histochemical fluorescence and the DA content of the neostriatum were markedly reduced. Removal of the neostriatum produced an increased fluorescence of the DA nerve cells of the substantia nigra. This is strong evidence for the existence of nigro-striatal DA neurons, which probably contain most or all of the DA present in the neostriatum (Anden et al., 1964). It has been suggested that the presence of DA in this structure is responsible for stereotyped behavior

and since APO has some structural similarity to DA (Ernst, 1969) it is able to stimulate DA receptors in the neostriatum and evoke stereotypy.

The mechanism by which APO induces stereotyped behavior in rats was demonstrated by Ernst (1967) to be the direct stimulation of DA receptors located in the neostriatum. Definitive evidence for a direct stimulation by APO of striatal DA receptors comes from the study of APO effects in rats pretreated intraventricularly with 6-hydroxydopamine (6-OHDA) (Schoenfeld and Uretsky, 1972). It was reported that the selective destruction of catecholamine-containing nerve terminals in the CNS with 6-OHDA caused an increase in sensitivity of rats to APO, so that the ED_{50} for stimulation was halved. These results are in agreement with Ungerstedt (1971) who found that in rats whose substantia nigra have been bilaterally destroyed with 6-OHDA, a given dose of APO produced a compulsive gnawing of greater intensity in the lesioned animals than in the intact ones. These results demonstrate that APO acts directly by stimulating DA receptors and that denervation induces a supersensitivity to its effects. In addition, rats pretreated with central DA-antagonists showed no signs of stereotyped behavior following a challenge dose of APO (Niemegeers and Janssen, 1979). This evidence supports the dopaminergic nature of this APO-induced effect.

Although it is currently accepted that neostriatal

dopaminergic systems are important for the development of stereotyped behavior patterns, previous studies have shown that after electrolytic brain lesioning of the nucleus amygdaloideus centralis (a very old nucleus in the archistriatum), the more intense aspects of APO stereotypy were abolished (Costall and Naylor, 1973a). In addition, there is evidence to suggest that the dopaminergic neurons of the mesolimbic system may also have a degree of involvement with stereotyped behavior. It was found that APO-induced stereotyped behavior was reduced when the ascending dopaminergic neurons to the mesolimbic system were lesioned. Lesioning of both the tuberculum olfactorium and nucleus accumbens abolished the sniffing component of APO-induced stereotyped behavior while lesion of the stria terminals was without effect upon APO stereotypy (Costall and Naylor, 1973a).

IV. Dopaminergic Involvement in Neuroleptic-Induced Catalepsy

When neuroleptics are given to animals, catalepsy results. In this state of sedation, the animals do not move spontaneously, and do not respond when they are placed in unusual postures or positions, e.g. on a tabletop with the front limbs elevated using a horizontal bar (Costall and Naylor, 1974a). On the other hand, they are not hypnotized (as they are with barbiturates, etc.), and their muscular power is retained (in contrast to animals sedated

with muscle relaxants such as meprobamate, chlordiazepoxide, etc.). The cataleptic animals react to painful stimuli and right themselves when placed on their backs (Munkvad et al., 1968). When low doses of the narcotic agonist, morphine, are given to animals, catalepsy occurs, providing the observed state of immobility is not associated with an increase in muscle tone greater than observed during neuroleptic catalepsy. However, if increasing the dose of the narcotic agonist causes an immobility associated with marked rigidity, this state is commonly termed catatonia (Costall and Naylor, 1974a).

Practically all neuroleptics produce in man a neurologic syndrome referred to as EPS. The majority of extrapyramidal reactions consist of "pseudo parkinsonism" (akinesia, muscular rigidity and tremor) and akathisia (a syndrome of motor restlessness). In drug-induced parkinsonism, the dominating symptom is usually akinesia. The akinetic syndrome produced in laboratory animals by neuroleptic drugs is referred to as catalepsy. When testing a series of compounds suspected of neuroleptic (extrapyramidal) potency, catalepsy is the most common test used for this type of evaluation. The clinical activity of the dopaminergic antagonists roughly correlates to their ability to induce catalepsy in experimental animals (Hornykiewicz, 1975). Although this pharmacological screening procedure holds true for most typical neuroleptic compounds, a number of atypical

neuroleptics (TRZ, clozapine and sulpiride) with antipsychotic potency similar to CPZ, were relatively weak inducers of catalepsy in rats or devoid of cataleptogenic properties altogether (Niemegeers and Janssen, 1979; Bürki et al., 1975).

There is neuropharmacologic evidence demonstrating that dopaminergic antagonists induce catalepsy by acting on the corpus striatum. Bilateral electrolytic lesions of the corpus striatum were observed to attentuate neuroleptic (CPZ)-induced catalepsy (Koffer et al., 1978). This is in agreement with Honma and Fukushima (1978) who found that bilateral lesioning of the striatum weakened the catalepsy induced by CPZ and TRZ.

The antiemetic compound, metoclopramide, which has been suggested to decrease striatal dopaminergic activity by blocking the striatal DA receptors, was found to produce a dose-dependent catalepsy in mice (Ahtee and Buncombe, 1974) and in rats (Ahtee, 1975). In both of these reports, the induction of catalepsy is associated with a five- to six-fold elevation of striatal homovanillic acid (HVA), one of the main metabolites of DA. This increased striatal HVA concentration without any decrease in DA content most probably indicates that DA formation is increased. APO reduced the catalepsy and the increase in striatal HVA concentration caused by metoclopramide in rats. These findings in conjunction with lesion studies previously mentioned suggest that neuroleptics produce catalepsy by blocking DA receptors in the striatum.

The relative importance of the mesolimbic system in neuroleptic catalepsy was investigated by placing a lesion at the rostral level of the hypothalamus to destroy only the ascending pathway to the mesolimbic areas. These studies indicate a critical role for the mesolimbic system in the mediation of neuroleptic catalepsy since the lesions caused a marked reduction of the cataleptic actions of spiroperidol, perphenazine, lenperone (LEN), and TRZ. It would, therefore, appear that not only extrapyramidal but also mesolimbic areas are important for the mediation of neuroleptic catalepsy (Costall and Naylor, 1973b). It was further determined that there is a differential involvement of mesolimbic areas with the behavioral effects of catalepsy. The cataleptic action of HAL and LEN was reduced by lesions of the nucleus accumbens and stria terminalis, however, ablation of the tuberculum olfactorium did not modify the cataleptic action of any agent tested (Costall and Naylor, 1974b). Even though evidence suggests the involvement of mesolimbic areas, Honma and Fukushima (1978) concluded that the striatum more than the nucleus accumbens is involved in producing catalepsy with neuroleptics, since lesions in the nucleus accumbens had fewer effects on catalepsy than did those in the striatum.

It would appear that cholinergic as well as dopaminergic mechanisms are important for the control of catalepsy since neuroleptic catalepsy can be enhanced or reduced by cholinergic or anticholinergic agents respectively (Morpurgo, 1962).

Central cholinergic stimulation by arecholine or pilocarpine has been found to induce catalepsy. The cataleptic activity of these drugs, like that of neuroleptics, was easily antagonized by anticholinergic agents, especially scopolamine (Zetler, 1971). It is suggested that the interaction between the two neurotransmitter systems may take the form of a reciprocal balance and that since the striatum is rich not only in DA but also in acetylcholine (ACh) then this may be a site for the balanced mechanisms (Costall and Naylor, 1974c).

The catalepsy and increased concentration of striatal HVA induced by neuroleptic drugs can be antagonized by anticholinergic drugs (Anden, 1974). There is also other evidence that ACh and DA have antagonistic actions in the striatum. It has been shown that pilocarpine increases the concentration of HVA in the striatum and this increase was antagonized by atropine but not by APO (Ahtee and Kaariainen, 1974). When rats were pretreated with alpha-methylparatyrosine (αMT), neuroleptic catalepsy was potentiated. It was, therefore, interesting that cholinergic catalepsy was also potentiated when catecholamine levels were reduced by αMT pretreatment, for this would suggest that a catecholaminergic system normally opposes the cholinergic induced effect and, thus, supports the concept of a cholinergic-catacholaminergic balance mediating catalepsy (Costall and Naylor, 1974c; Ahtee and Kaariainen,

1974). Further conceptualization of this ACh-DA balance by Gianutsos (1979) is based on a consideration of a reciprocal balance in the striatum between ACh and DA where dopaminergic stimulation inhibits cholinergic neuronal activity as mentioned previously. Catalepsy may be considered as resulting from a net increase in stimulation of cholinergic receptors in the striatum. Cholinomimetics would then produce catalepsy by directly stimulating these receptors while CPZ would produce catalepsy by blocking DA receptors and disinhibiting cholinergic neurons leading to an increase in the efflux of ACh.

As to the mechanism of catalepsy, support for this ACh-DA balance in the striatum was offered by Honma and Fukushima (1978). They showed that bilateral electrocoagulation of rat striatum decreased neuroleptic-induced catalepsy. If electrocoagulation destroyed not only DA neurons but also cholinergic neurons, then complete destruction of dopaminergic-cholinergic connections would cause a reduction in the cataleptogenic activity of HAL. On the other hand, it was also shown that microinjection of 6-OHDA into the striatum gradually enhanced the catalepsy induced by HAL suggesting that removal of the inhibitory activity of dopaminergic neurons results in an excitation of cholinergic neurons. When Costall and Naylor (1973b) destroyed the dopaminergic pathways which supply the extrapyramidal brain regions by lesioning the lateral hypothalamus, they found that neuroleptic catalepsy

was enhanced. If the lesion caused dissruption to the dopaminergic component of the postulated dopaminergic-cholinergic balance operating via the extrapyramidal nuclei, cholinergic dominance would result. These studies offer support for the involvement of an intrastriatal balance between dopaminergic and cholinergic neurons in neuroleptic-induced catalepsy.

V. Dopaminergic Involvement of Apomorphine-Induced Hypothermia in Rat

Since the introduction of specific DA agonists and antagonists, evidence has accumulated to suggest that stimulation of central DA receptors can bring about thermoregulatory changes in several species. In the cat, the dose-related fall in body temperature seen after intraventricular injection of DA was blocked by HAL (Kennedy and Burks, 1974). In mice, systemically administered APO produced a dose-related lowering of body temperature. This hypothermic effect is competitively antagonized by HAL, pimozide, spiroperiodol, and perphenazine (Fuxe and Sjöqvist, 1972; Barnett et al., 1972; Buus Lassen, 1974). APO has been consistently reported to cause a decrease in the core body temperature of the rat (Kruk, 1972; Ary et al., 1977; Cox et al., 1978). These studies also show that pimozide or HAL can block the hypothermic action of APO. It appears that both DA and APO mediate hypothermic responses through activation of DA receptors.

The site of action of APO has been demonstrated to reside within the preoptic-anterior hypothalamus of the rat (Cox and Lee, 1977b, 1979; Cox et al., 1978; Colbec and Costentin, 1980). Administration of either DA or APO into the preoptic-anterior hypothalamus caused an almost immediate rise in tail skin temperature and a concomitant fall in core temperature in the rat (Cox et al., 1978). Following the systemic injection of pimozide, the dose-dependent hypothermic response induced by intrahypothalamic DA or amphetamine was significantly reduced (Cox and Lee, 1979).

It has been reported that tolerance to the hypothermic action of APO can occur. Chiel et al. (1974) have shown that when rats are tested daily with intraperitoneal injections of APO, the hypothermic response of subsequent injections of APO is reduced. It was suggested that a decreased sensitivity of the DA receptor was responsible for the tolerance to the hypothermic effects of APO. In addition, it was found that tolerance to the hypothermic effects of APO developed in rats following repeated injections of APO in the rostral hypothalamus at the preoptic-anterior hypothalamic nuclei (Ary et al., 1977). This same study showed that although injections of APO in the area surrounding the lateral ventricle did induce hypothermia, tolerance to the hypothermic effects of APO was not observed. It was therefore proposed that the hypothermic

effects of APO are mediated via two different types of DA receptors at two different sites: 1) in the rostral hypothalamus where the receptors develop tolerance; and 2) in areas surrounding the lateral ventricle, where the receptors are resistant to tolerance (Ary et al., 1977).

There is considerable evidence to support the concept that ACh plays a role in central thermoregulatory mechanisms. Iontophoretic injection of ACh into the rostral hypothalamic thermoregulatory centers causes a fall in body temperature in the rat (Kirkpatrick and Lomax, 1970) and this response is abolished by prior systemic injection of atropine indicating a specific effect at cholinergic receptor sites. Direct injection of atropine into the rostral hypothalamus of the rat produces hyperthermia, presumably due to blockade of central cholinergic activity (Kirkpatrick and Lomax, 1967).

The interactions between the cholinergic and dopaminergic systems in thermoregulation have been studied by Glick and Marsanico (1974). In their studies, it was found that both APO and pilocarpine induced a dose-dependent hypothermia in mice. In addition, when administered together, the hypothermic effect was greater following a combination of the drugs than following either drug alone. In the animals treated with APO, either HAL or scopolamine were able to antagonize the hypothermic effect, whereas only scopolamine antagonized pilocarpine-induced hypothermia. The authors suggest that mechanisms involving

both dopaminergic and cholinergic neuronal systems contribute to temperature regulation. These data conflict to temperature regulation. These data conflict with Cox and Lee (1977a) whose studies showed no evidence to support the hypothesis that APO-induced hypothermia involves a cholinergic link. They found that the anticholinergic drugs, scopolamine and atropine were highly effective antagonists of oxotremorine-induced hypothermia, but in the same doses they were ineffective against the hypothermic effect of APO.

VI. Evidence to Suggest Multiplicity of Dopamine Receptors in the Central Nervous System

Over the last ten years, experience with DA has led investigators to consider the DA receptor as something other than a single entity. Like other receptor systems, the DA receptor has been characterized and subdivided into numerous subtypes. In the present review, the contention that multiple DA receptor types exist in the CNS will be supported by the available evidence.

In studying the pharmacology of tardive dyskinesias, Klawans (1973) found that tardive dyskinesias can occur independently of drug-induced parkinsonism and suggested that there are two separate populations of DA receptors in the striatum which are differentially susceptible to neuroleptic blockade. This finding as well as others stimulated researchers in psychopharmacology to further examine the

the facilitory and inhibitory nature of DA receptor types.

In 1976, Cools and Van Rossum (1976) hypothesized the existence of multiple DA receptor types in mammalian nervous tissue. On the basis of their critical analysis, they concluded that there exists two electrophysiologically distinct types of DA sensitive units, which are each characterized by their own topographical, pharmacological, biochemical and ontogenic features. Histochemical experiments on the rat provide evidence that there exists two distinct types of DA-terminal structures in the brain. In the neostriatum and nucleus accumbens were found structures marked by diffuse green DA-fluorescence and structures marked by dotted DA-fluorescence respectively. It was suggested that these DA-loaded structures within the brain contain two populations of DA receptors: excitatory-mediating DA receptors (DA_e), found in the neostriatum and inhibitory-mediating DA receptors (DA_i), found in the nucleus accumbens. The DA_e-structures not only appear later in postnatal development than the DA_i-structures, but also are more sensitive to depletion by αMT and have a higher turnover rate (Cools and Van Rossum, 1976). It was also suggested by Cools and Van Rossum (1976) that the DA_e-receptors are located presynaptically, since both APO and HAL are able to interact with the same presynaptic DA receptor (Christiansen and Squires, 1974; Costall and Naylor, 1973c; Seeman, 1974).

The general feeling that there may exist more than one type of DA receptor has been explored along a number of lines. Thus, in gallamine-immobilized cats, the caudate nucleus and the nucleus accumbens were perfused by means of a push-pull cannula and DA was measured in the perfusate. Following probenicid administration, the net effect of sulpiride, clozapine and TRZ on HVA accumulation was more marked in the limbic system than in the striatum whereas HAL and CPZ had a similar effect in the two regions (Bartholini, 1976). Uzan et al. (1978) found similar results in their work with mezilamine, a relatively new antipsychotic agent related to the atypical neuroleptics. In addition to producing a greater accumulation of HVA in the mesolimbic area than in the striatum of the rat, mezilamine also showed weak cataleptogenic properties. This possibly accounts for the fact that atypical neuroleptics display an antipsychotic action and yet cause less EPS than HAL and CPZ.

The accumulation of HVA in rodent brain by neuroleptics is thought to be mediated by a neuronal feedback mechanism triggered by the blockade of postsynaptic DA receptors (Da Prada and Pletscher, 1966; Roos, 1969). If this is so, then the DA receptors located in the striatum and mesolimbic system should be selectively blocked by the classical and atypical neuroleptics, respectively. To test this hypothesis, Le Fur et al. (1980) studied the in vivo

displacement of the binding of ^{3}H-spiroperidol (a high affinity ligand for the DA receptor) by classical and atypical neuroleptics. In this study, no correlation between regional stimulation of DA turnover and the selective blockade of DA receptors was found.

Although Le Fur et al. (1980) were unable to demonstrate a selective blockade of dopaminergic receptors in the striatum by the classical neuroleptics or in the mesolimbic system by the atypical neuroleptics, the behavior studies of Ljungberg and Ungerstedt (1978) were able to support the hypothesis that there exist different DA receptor types in the limbic and striatal areas of the brain with differing sensitivities to antagonist action. They examined whether different types of neuroleptic drugs with different clinical profiles may differ in their ability to antagonize two different APO-induced behaviors, one characterized by compulsive gnawing and another characterized by increased locomotion, and whether a difference in their specificity would relate to their ability to induce EPS. It was found that classical neuroleptic drugs causing high incidence of EPS (metoclopramide, HAL, CPZ) predominantly antagonized the APO-induced gnawing, while the atypical neuroleptic drugs (clozapine, sulpiride, TRZ), showing antipsychotic potency together with low incidence of EPS, instead antagonized the APO-induced locomotion (Ljungberg and Ungerstedt, 1978).

Biochemical and electrophysiological studies have provided evidence for a new dopaminergic receptor whose function seems to be the regulation of DA influence on postsynaptic cells. The presynaptic neuron itself might contain DA autoreceptors because (1) DA and APO can block DA synthesis in synaptosomal fractions of rat striatum, and this effect is reversed by neuroleptics (Christiansen and Squires, 1974) and (2) microiontophoretic application of DA or APO directly upon the soma of DA neurons located in zona compacta of substantia nigra decreased the firing of these cells, and this effect could be blocked by neuroleptic drugs (Aghajanian, 1977). These data are consistent with the observation that low doses of APO are able to depress locomotor function, which is considered to result from a stimulation of presynaptic receptors at doses marginally lower than those required to stimulate postsynaptic receptors (Di Chiara et al., 1976). In addition, binding studies indicate that ^{3}H-apomorphine has a prediliction for presynaptic DA receptors (Nagy et al., 1978). The authors observed that lesioning with 6-OHDA resulted in a selective and extensive retrograde loss of DA neurons in the substantia nigra, reducing ^{3}H-apomorphine binding in this structure by 76%. This data provides direct evidence for the presence of DA receptors on DA terminals or axons in the striatum. Furthermore, from binding studies of ^{3}H-neuroleptics and ^{3}H-apomorphine in schizophrenic brains, it was hypothesized that ^{3}H-apomorphine and ^{3}H-haloperidol

may be measuring preferentially pre- and postsynaptic DA receptors, respectively (Lee et al., 1978). This is consistent with the observation that low doses of APO do not exacerbate but may alleviate some psychotic symptoms (Angrist et al., 1975). These data indeed suggest that DA neurons themselves contain receptors for DA.

Although the application of receptor labelling assays has proved useful for many pharmacologists in receptor identification and localization, the interpretation of data from in vitro binding studies requires great caution. Most authors agree that the critical difficulty in labelling of receptors with radioactive ligands is that such binding may reflect binding to non-specific sites and that sometimes, drugs can displace at non-specific binding sites. For this reason, perhaps the most important criteria to fulfill in order to assess the specificity of binding is drug displacement, necessitating the use of a large number of antagonists and agonists belonging to different chemical classes (Laduron, 1980). With regard to the central DA receptor, ^{3}H-haloperidol (Creese et al., 1975), ^{3}H-pimozide (Baudry et al., 1977), ^{3}H-domperidone (Baudry et al., 1979), and ^{3}H-sulpiride (Theodorou et al., 1979) have been used as antagonist ligands, whereas ^{3}H-dopamine (Creese et al., 1975), ^{3}H-apomorphine (Seeman et al., 1976) and ^{3}H-2-amino-6,7-dihydroxytetrahydronaphthalene (ADTN) (Seeman et al., 1979) are the most important agonist ligands. It has been suggested that the DA receptor can exist in two

interconvertible conformations: the antagonist form and agonist form, since DA antagonists and agonists preferentially displace the antagonist ligands and agonist ligands respectively (Snyder, 1975). Significance of this agonist-antagonist receptor state must remain speculative since together with the fact that APO has a relatively high affinity for the HAL binding sites, a newly available ^{3}H-ligand (^{3}H-N-propyl-norapomorphine) which can be selectively directed to either of the two DA receptors has been reported (Titeler and Seeman, 1979).

In addition to the study of the binding of DA agents to putative DA receptors, an attempt to increase the knowledge about the nature of DA receptors has led investigators to study the DA receptor of neural tissue associated with a DA-sensitive adenylate cyclase. In the early seventies, Greengard's group raised the possibility that the therapeutic effects, as well as the extrapyramidal side effects, of antipsychotic agents may be attributable, at least in part, to their ability to block the activation by DA of specific DA-sensitive adenylate cyclases in the human brain (Clement-Cormier et al., 1974). Since then, characterization of this enzyme from the caudate nucleus of rat brain (Clement-Cormier et al., 1975) as well as geometric and stereospecific requirements of antagonists of the DA receptor-cyclase complex (Clement-Cormier et al., 1979) have been reported. By means of differential and density

gradient centrifugation procedures, this DA-sensitive adenylate cyclase has been regarded as a marker enzyme of the postsynaptic membrane (Laduron et al., 1976).

It is evident that DA receptors can be divided by biochemical or pharmacological criteria. These criteria can also be used to separate those DA receptors which are linked to the enzyme adenylate cyclase and those which are not. The bovine parathyroid provides an example of a type of DA receptor which stimulates the enzyme adenylate cyclase, whereas the mammatrophs of the anterior pituatary contain DA receptors which do not stimulate adenylate cyclase (Kebabian and Calne, 1979). It was proposed that the DA receptors in these two tissues are examples of two distinct categories of receptor designated by Kebabian and Calne (1979) as D-1 and D-2 respectively.

As an example of the location of different DA receptors within a tissue, it is of interest to examine the striatum, the most extensively studied dopaminergic brain region. Destruction of the nigro-striatal neurons with 6-OHDA lesions causes no loss of either striatal (Krueger, 1976) or nigral (Kebabian and Saavedra, 1976) DA-sensitive adenylate cyclase activity. Conversely, intrastriatal injection of kainic acid, a rigid glutamic acid analogue, destroys neuronal cell bodies in the striatum but spares the dopaminergic nerve terminals; this procedure causes a substantial loss of striatal DA-sensitive adenylate cyclase

activity but does not diminish the content of DA (Di Chiara et al., 1977; Hattori and McGeer, 1977). In summary, neither the autoreceptors of the cell bodies in the nigra nor the autoreceptors of the nigro-striatal terminals appear to be associated with a DA-sensitive adenylate cyclase.

Although a DA-sensitive adenylate cyclase has been identified in the substantia nigra as well as the striatum (Kebabian and Saavedra, 1976), the enzyme occurs on cellular elements other than the dopaminergic neurons. Recent studies with kainic acid lesions (to remove post-synaptic DA receptors) and ablations of the cerebral cortex provide further evidence to distinguish some of the specific binding sites from the DA-sensitive adenylate cyclase in the striatum (Schwarcz et al., 1978a). These experiments imply that the striatal DA-sensitive adenylate cyclase is located predominantly on neurons intrinsic to the caudate; in contrast, most of the striatal HAL binding sites are located on nerve terminals derived from neurons in the cerebral cortex.

In addition to the D-1 receptors present in the striatal region, it has been suggested that the DA receptors in guinea pig and carp retinas are all linked to adenylate cyclase (Watling et al., 1979). In order to study these retinal DA receptors, Watling et al., (1979) utilized ^{3}H-domperidone which appears to be a novel ligand (Baudry et al., 1979) specific for D-2 receptor binding sites in

vitro (Laduron and Leysen, 1979). Since a failure to observe specific binding of ^{3}H-domperidone to these tissues was noted, it implies they contain an apparent homogeneous population of D-1 receptors.

Recent data indicate that both D-1 and D-2 receptors exist in the anterior pituitary (Ahn et al., 1979). Thus, the adenylate cyclase-linked D-1 anterior pituitary receptor is not associated to prolactin release, but guanosine triphosphate (GTP) can modulate DA agonist binding to it (Sibley and Creese, 1979). On the other hand, the D-2 receptor located on the mammatrophs controls prolactin release (Caron, 1978), but GTP does not modulate the affinity of DA agonists for this site (Sibley and Creese, 1979). Guanine nucleotides also modulate DA agonist binding to D-1 receptors on striatal cell bodies (Creese et al., 1979b). This latter finding is based on the observation that destruction of striatal cell bodies with kianic acid causes both the loss of DA activated cyclase (Schwarcz et al., 1978a) and the loss of the ability of GTP to alter DA agonist displacement of ^{3}H-spiroperidol in striatal tissue (Creese et al., 1979a). Thus, GTP can modulate DA agonist binding to D-1 receptors in both the striatum and the anterior pituitary.

Considering the great variety of criteria that are used to characterize and subdivide DA receptors into subtypes, integrating the available data into one unifying concept has become virtually impossible. Although a unified

classification of central DA receptors has not yet been developed, the available evidence supports the concept of multiple DA receptor types in mammalian CNS.

Statement of the Problem

Current therapy of schizophrenia involves the use of neuroleptic agents which are believed to exert their biological activity through the blockade of DA receptors in the mesolimbic DA tract. In addition, these drugs block DA receptors in the corpus striatum and thus cause side effects resulting from impaired DA transmission in the extrapyramidal system. Neuroleptics also block DA-induced inhibition of prolactin release as well as other DA-regulated nervous functions in the central nervous system. The therapeutic problems arising from the non-selectivity of currently available drugs towards DA receptors could be eliminated, and major advancements into our understanding of neurological function and pathophysiology could be made if isoreceptors for DA were demonstrated. That such isoreceptors for DA actually exist may be actual fact; however, identification and pharmacological differentiation of these DA receptors remains a challenging task.

The present study was undertaken to further support the hypothesis that there might be multiple receptor types for DA in mammalian CNS tissue. A series of neuroleptic

compounds shall be subjected to a battery of whole animal screening procedures in an effort to demonstrate gross differences in potency of activity. These pharmacological tests shall include antagonism of APO-induced locomotor stimulation, stereotyped behavior and thermotropic action in rats and the induction of catalepsy by the neuroleptic drug. Dose response as well as time-activity relationships shall be determined.

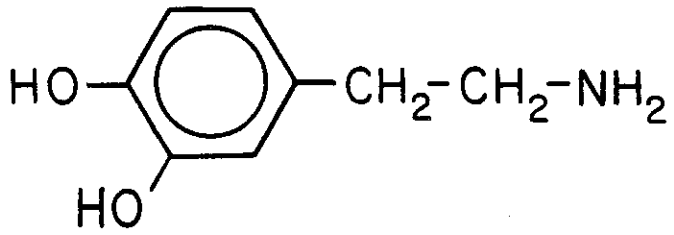
MATERIALS AND METHODS

Animals

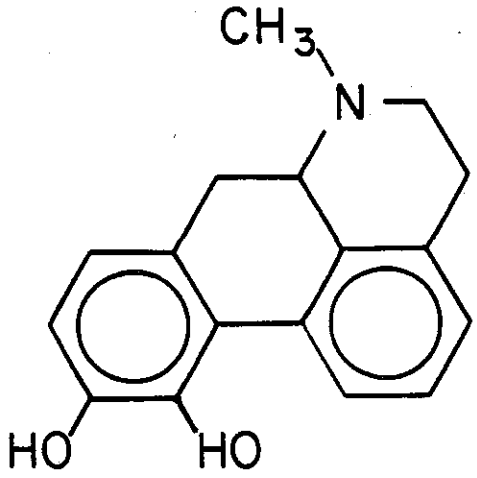
Adult male rats of the Wistar strain from Simonsen Laboratories (Gilroy, California), weighing 150-250g, were used in all experiments. On arrival, animals were housed in metal cages (50 x 35 x 20cm) in groups of 15 and exposed to a fixed light/dark cycle for a period of not less than one week prior to experimentation (0800-2000:light; 2000-0800:dark). Animals were offered a diet of commercially prepared pellets from Ralston Purina Company (St. Louis, Missouri) and tap water ad libitum until commencement of each experiment, at which time all food and water were withdrawn. All experiments were conducted in temperature-controlled facilities maintained at 23±1°C.

Drugs

Apomorphine hydrochloride (APO) obtained from Merck and Co. (Rahway, New Jersey) was employed as the dopamine (DA) agonist at a standard challenge dose of 2.0 mg/kg. Four neuroleptic compounds were also employed in this study. From the phenothiazine class, chlorpromazine hydrochloride (CPZ) was obtained from Smith Kline & French Laboratories (Philadelphia, Pennsylvania) and thioridazine hydrochloride

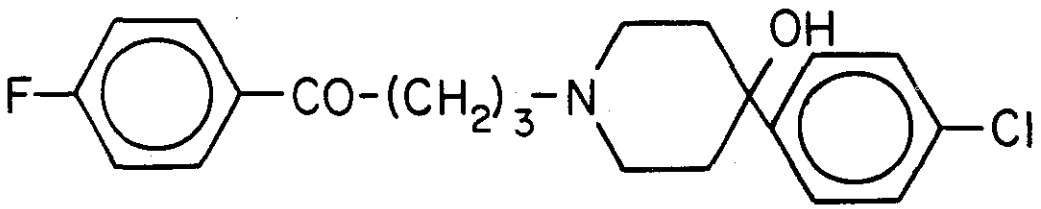


DOPAMINE mol wt 153.18

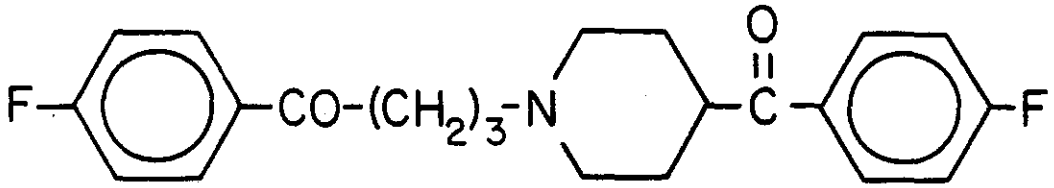


APOMORPHINE mol wt 267.31

Figure 2. Structural formulas of Dopamine and the dopamine agonist, Apomorphine.

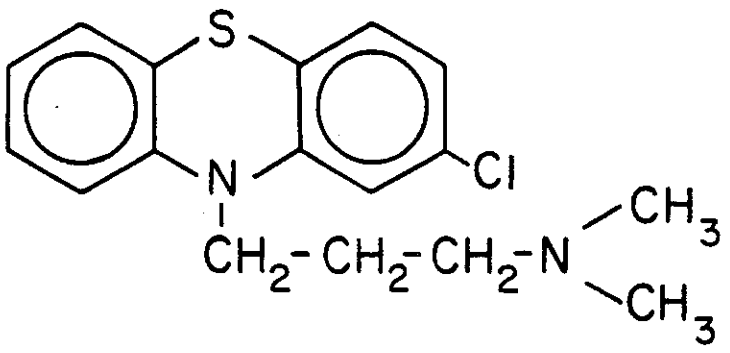


HALOPERIDOL mol wt 375.88

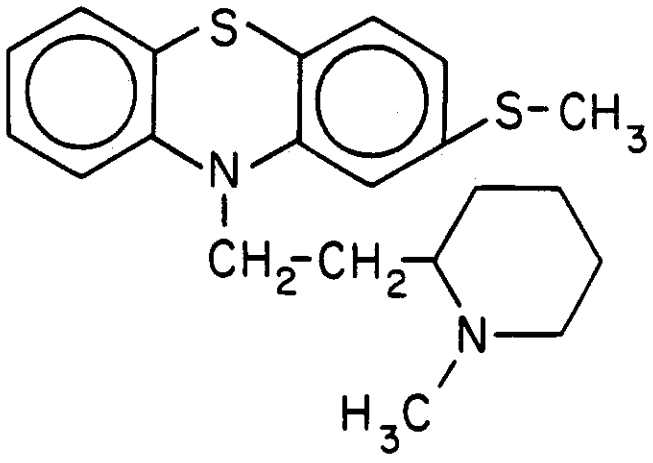


LENPERONE mol wt 371.43

Figure 3. Structural formulas of two butyrophenones, Haloperidol and Lenperone.



CHLORPROMAZINE mol wt 318.88



THIORIDAZINE mol wt 370.56

Figure 4. Structural formulas of two phenothiazines, Chlorpromazine and Thioridazine.

(TRZ) from Sandoz Pharmaceuticals (East Hanover, New Jersey). From the butyrophenone class, haloperidol lactate (HAL) ampules were obtained from McNeil Laboratories (Fort Washington, Pennsylvania) and lenperone hydrochloride (LEN) from A. H. Robins Company (Richmond, Virginia). In all experiments, each rat was treated with one of three logarithmically-spaced doses of either HAL, LEN, CPZ, or TRZ. Double-distilled water was used as a control.

All drug solutions were prpeared within 1 hour of injection using double-distilled water except for APO, which oxidizes in aqueous solution and was thus prepared within one minute of injection. The drugs were all administered intraperitoneally into the lower left quadrant of the abdomen using, a 1-ml Tuberculin syringe with a 5/8-inch, 25-gauge needle. To minimize volume effects, all drugs were administered in a constant volume of injection of 1.0 ml/kg.

Locomotor Activity

Locomotor activity was measured in a small soundproofed diffusely illuminated room, using a Model "S" Selective Activity Meter with printing counter manufactured by Columbus Instruments (Columbus, Ohio). Groups of three randomly chosen rats were placed in a clear plastic cage with bedding material (45 x 24 x 20cm) and positioned on the activity monitor at approximately 1200 hours (Plate 1). At 1530 hours, the three rats were pretreated with either a neuroleptic or

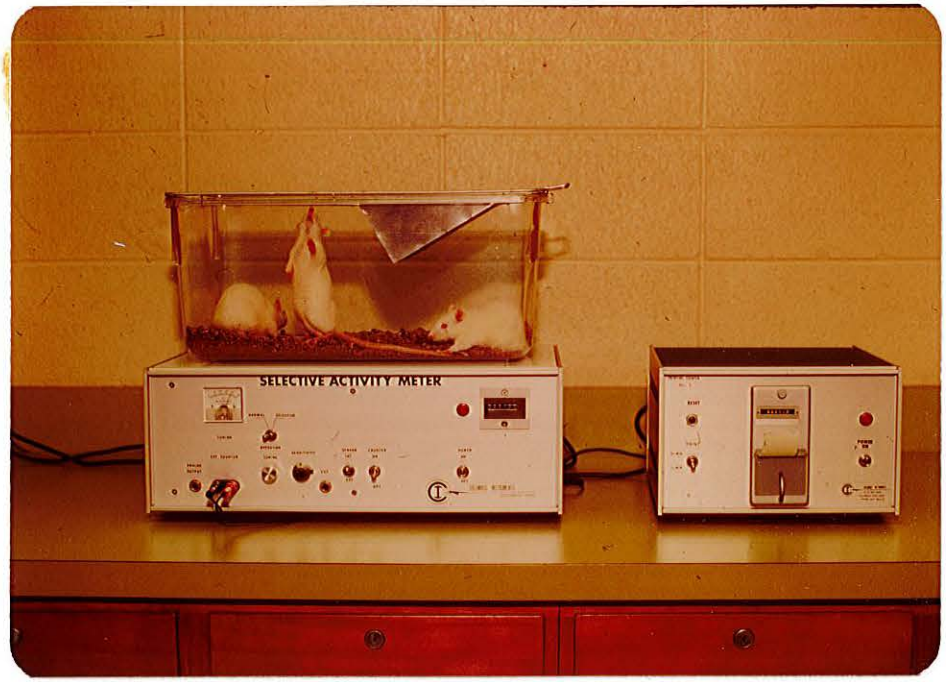


Plate 1. Experimental setup for measuring Locomotor Activity

Plate 2. Experimental setup for observing Stereotyped Behavior

double-distilled water. Thirty minutes later, each rat was treated with APO at which time monitoring of the group locomotor activity commenced. Locomotor activity was measured by the monitor every 6 minutes and the total locomotor activity score determined for the first 60 minutes following APO injection.

Stereotyped Behavior

Stereotyped behavior was assessed while the animals were individually housed in clear plastic cages (45 x 24 x 20cm) separated by cardboard screens in a diffusely illuminated room (Plate 2). Experiments were conducted between 1200 and 1600 hours. The experimental animals were allowed to acclimate to their environment for at least 1 hour prior to commencement of the experiment. At 1200 hours, the rats were pretreated in a double-blind fashion with one of the four neuropletic drugs or double-distilled water; 30 minutes later, all animals were challenged with APO. Thereafter, stereotyped behavior was scored at 5-minute intervals for 90 minutes using the scoring system summarized in Table I. The individual score for each animal over the 90-minute period was the sum of the 18 5-minute readings.

Catalepsy

For measurement of catalepsy, the test animals were randomly placed in individual clear plastic cages (45 x 24 x

Table I. Scoring System for Estimation of the Intensity of Stereotyped Behavior.

Assigned Score	Behavior Displayed
1	Behavior indistinguishable from that displayed by untreated animals.
2	Periodic sniffing, and/or repetitive head and limb movements.
3	Continuous sniffing with exploratory activity, and repetitive head and limb movements.
4	Occasional or periodic gnawing, biting, or licking, and exploratory activity with continuous sniffing.
5	Persistent and intense gnawing, biting, or licking with locomotion restricted to a small area.
6	Compulsive gnawing, biting, or licking at one location.

Martin and Quock, 1978.

20 cm) in a diffusely illuminated room. Each cage was screened from the others by cardboard screens. Each rat was placed in a cage 1 hour prior to drug treatment to allow adaptation to the new environment. All observations were made between 1200 and 1600 hours. The observation table was equipped with a 10-cm high horizontal bar adjacent to the cages and blocked from view with carboard screens (Plate 3). Following treatment with one of the four neuroleptic drugs or double-distilled water, each animal was tested every 15 minutes for catalepsy by placing both front limbs over the bar. A cataleptic animal will maintain this position for various periods of time dependent upon the degree of catalepsy. The moment the animal moved off the bar with both front limbs, the reading from the stop watch used to measure each animal's duration was then recorded. Each animal was placed on the bar two times at each 15-minute interval within the 90-minute test period to determine an average duration in seconds. In order to account for animals maintaining the imposed position for an infinite period of time, a maximum duration of 120 seconds was allowed for any observation. The total bar time (in seconds) for each animal tested was the sum of six 15-minute readings, each of which represent the average of two test runs.

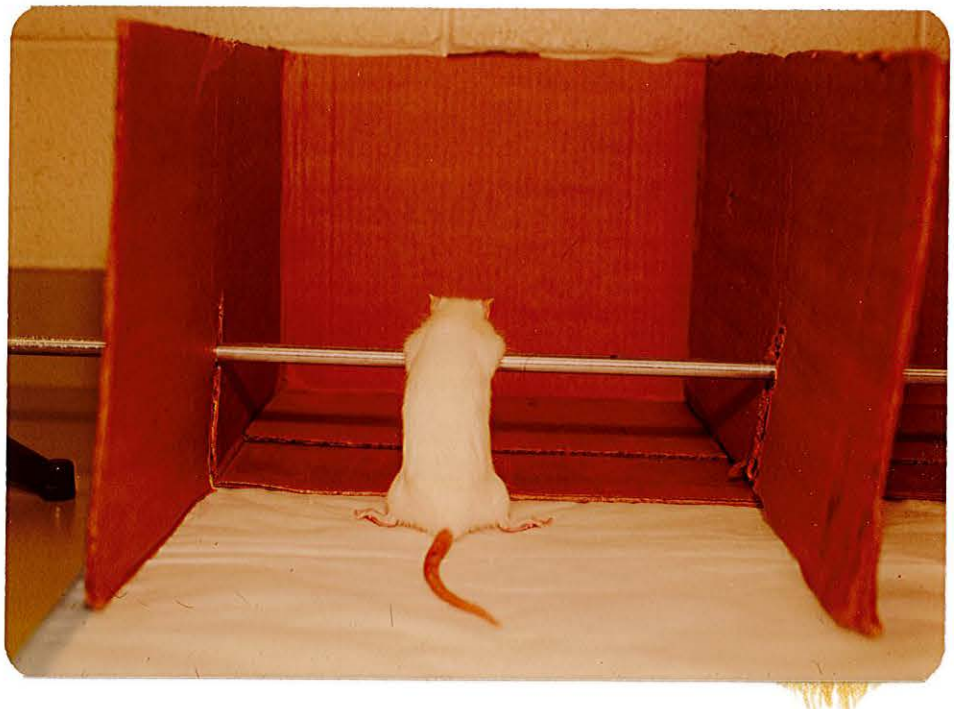


Plate 3. Experimental setup for measuring the degree of Catalepsy

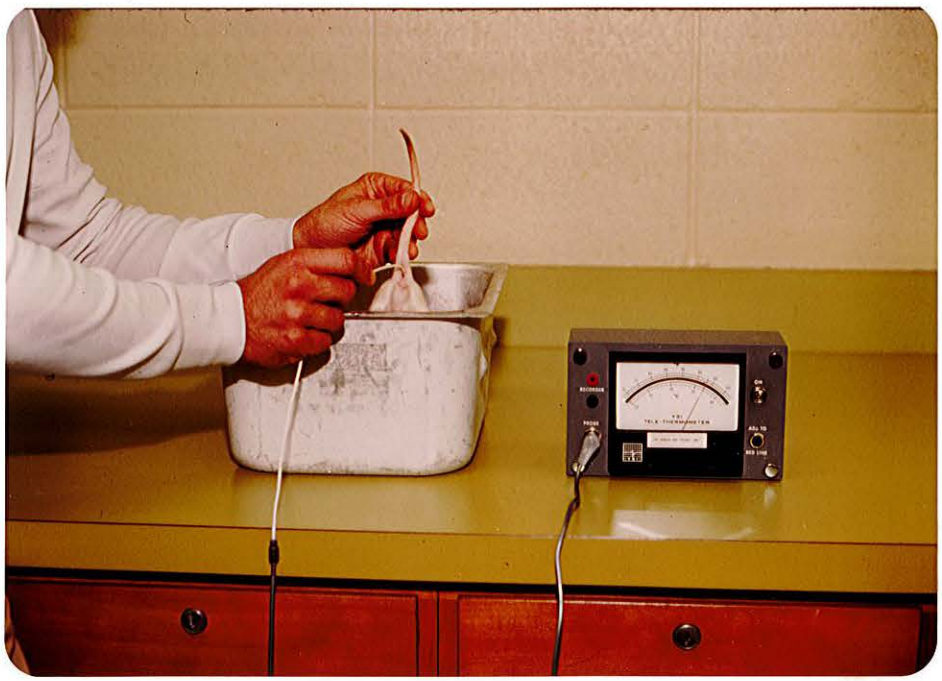


Plate 4. Experimental setup for measuring Body Temperature

Body Temperature

Core temperature was measured with a Yellow Springs Tele-Thermometer (Model 43TA) and thermistor probe inserted approximately 4 cm. through the rectum while the animals were individually housed in metal cages (24 x 30 x 15cm) (Plate 4). Experiments were conducted between 1200 and 1600 hours. The experimental animals were allowed to acclimate to the testing environment for at least 1 hour prior to the commencement of the experiment at which time baseline body temperatures were measured at 15-minute intervals until consistent readings ($\pm$ 0.2^{o}C) were obtained. The test animals were then pretreated with either a neuroleptic drug or double-distilled water 30 minutes prior to challenge with either APO or double-distilled water. The rectal temperature was then measured every 15 minutes for the duration of the expected hypothermia, i.e. 90 minutes.

Statistics

The data obtained for the following time activity relationships: 1) the locomotor activity counts for the 60-minute scoring period; 2) the cumulative stereotyped behavior scores for each 15-minute interval; 3) the duration of catalepsy for each 15-minute interval; and 4) the change in body temperature for each 15-minute interval, were averaged and compared between rats treated with one of three logarithmically-spaced doses of either HAL, LEN, CPZ or

TRZ and the double-distilled water control using a Friden 1155 Calculator programed for Dunnett's t-test.

The molar ED_{50} of each neuroleptic used in this study for each of the pharmacological parameters was determined with the use of a Hewlett Packard 25C Calculator. This calculator was programed for linear regression analysis in order to obtain values for y-intercept and slope. In order to take into account the molecular weight of each neuroleptic used, throughout all calculations, the dose of each neuroleptic was expressed as the log mole dose. The raw data obtained for each dose of neuroleptic tested in all parameters was expressed as a percentage value. In the locomotion studies, the locomotor activity count for each 60-minute scoring period was expressed as the percentage of maximum inhibition of locomotor activity. In the stereotyped behavior studies, the total stereotyped behavior score for each animal over a 90-minute period was expressed as the percentage of maximum inhibition of stereotyped behavior. In the catalepsy studies, the total bar time for each animal tested was expressed as the percentage of maximum bar time (720 seconds). In the body temperature studies, the maximum drop in body temperature over the 90-minute testing period for each animal was expressed as the percentage of maximum inhibition of hypothermia.

Within each parameter tested, the log molar ED_{50} for each neuroleptic was determined by entering each of three

log mole doses of a particular neuroleptic into the calculator as the x-values along with their respective percentage figures representing the y-values. By determining the equation of the line, the log molar ED_{50} for HAL, LEN, CPZ and TRZ within each parameter was calculated by entering 50 as the y-variable and solving for the x-variable. The 95% confidence limits were also calculated for each mole ED_{50} value.

The log molar ED_{50} of each neuroleptic used in this study for each of the pharmacological parameters were analyzed quantitatively by means of regression analysis. Each parameter was paired with each of the others, making a total of 6 pairings. Within each pairing, the log molar ED_{50} for HAL, LEN, CPZ and TRZ of one parameter was entered into the Hewlett Packard 25C as the x-values and the log molar ED_{50} for HAL, LEN, CPZ and TRZ for the second parameter was entered as the y-values. A correlation coefficient was determined for each pairing as well as the line of best fit (Table X). This same method of analysis was used in determining the correlation coefficient and equation of the line for each of the 6 pairings of parameters with TRZ excluded from the analysis (Table XI).

RESULTS

1. Locomotor Activity

APO 2.0 mg/kg produced a significant increase in locomotor activity when injected intraperitoneally into rats (Figure 5) compared to a vehicle injection of double-distilled water. Vehicle-injected rats also showed no characteristics of stereotyped behavior whatsoever. Pre-treatment with three logarithmically spaced doses of HAL, LEN, CPZ or TRZ produced a dose-related antagonism of the increase in locomotor activity induced by APO 2.0 mg/kg with significant antagonism exhibited by HAL at a dose of 0.1 mg/kg (Figure 6), LEN at a dose of 0.3 mg/kg (Figure 7), CPZ at a dose of 2.0 mg/kg (Figure 8), and TRZ at a dose of 8.0 mg/kg (Figure 9).

2. Stereotyped Behavior

APO 2.0 mg/kg induced stereotypy when injected intraperitoneally into rats. During the 90-minute scoring period the rats initially displayed a low intensity of stereotyped behavior consisting of periodic to continuous sniffing and repetitive head and limb movements. Within 30 minutes this progressed to a full blown stereotypy consisting of gnawing, biting or licking at one location which frequently lasted several minutes without interruption.

Table II. The effect of pretreatment (-30 minutes) with either haloperidol lactate, lenperone hydrochloride, chlorpromazine hydrochloride or thioridazine hydrochloride on the locomotor activity effects of intraperitoneal apomorphine hydrochloride over a 60-minute period in male rats.

Drug	Dose	N	Locomotor Activity Counts	% of Maximum Inhibition of Locomotor Activity
Apomorphine	2.0	6	581.33 ± 208.61	
Haloperidol	0.03	6	418.67 ± 132.41	27.98 ± 22.78
	0.06	6	237.83 ± 58.47	59.09 ± 10.06
	0.1	6	63.0 ± 15.01	89.16 ± 2.58
Lenperone	0.03	6	409.50 ± 126.33	29.56 ± 21.73
	0.1	6	206.33 ± 71.96	64.51 ± 12.38
	0.3	6	43.17 ± 12.45	92.57 ± 2.14
Chlorpromazine	0.5	6	577.50 ± 157.71	0.66 ± 27.13
	1.0	6	219.0 ± 82.58	62.33 ± 14.20
	2.0	6	93.0 ± 25.55	83.86 ± 4.40
Thioridazine	2.0	6	521.33 ± 146.33	10.32 ± 25.17
	4.0	6	311.0 ± 60.95	46.50 ± 10.48
	8.0	6	59.33 ± 24.20	89.79 ± 4.16

Dose is expressed as mg/kg. Values are reported as the mean ± 1.0 SEM.
N represents the number of groups used. Each group contains 3 rats.

Figure 5. The mean effects of intraperitoneal apomorphine hydrochloride or double-distilled water on the locomotor activity of male rats pretreated (-30 minutes) with double-distilled water (vertical bars indicate 1.0 SEM).

Key: H20/H20 = Double-distilled water 1.0 ml/kg followed by double-distilled water 1.0 ml/kg (N=6)

H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCL 2.0 mg/kg (N=6)

* = Using Students t-test, the locomotor activity of rats treated with APO 2.0 were significantly different from the locomotor activity of rats treated with double-distilled water ($P < 0.05$)

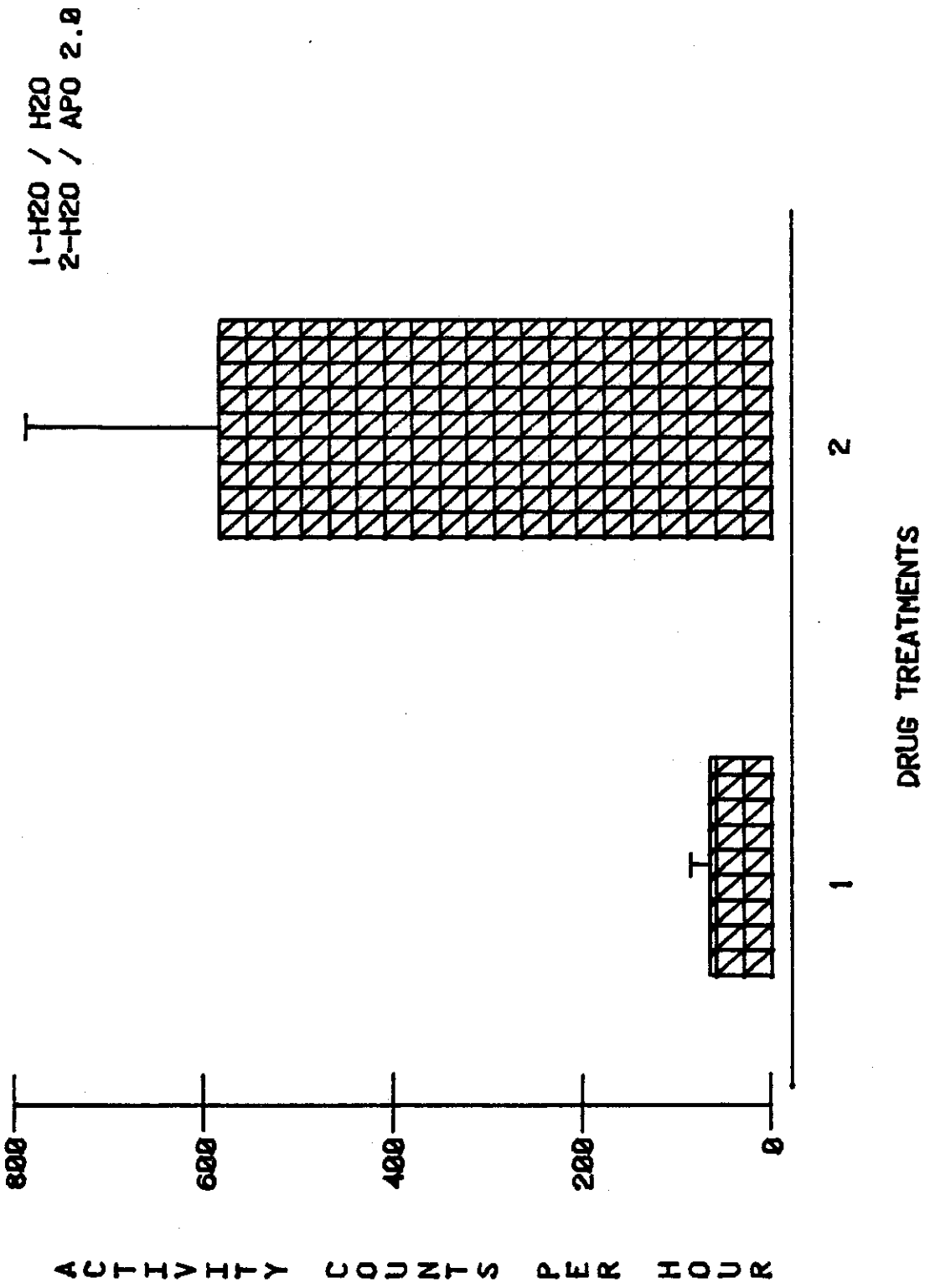

1-H20 / H20
2-H20 / APO 2.0
800
600
400
200
0
1
2
DRUG TREATMENTS
ACTIVITY COUNTS PER HOUR

Figure 6. The mean effects of pretreatment (-30 minutes) with haloperidol lactate on the locomotor activity effects of intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

HAL 0.03/APO 2.0 = Haloperidol lactate 0.03 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

HAL 0.06/APO 2.0 = Haloperidol lactate 0.06 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

HAL 0.1/APO 2.0 = Haloperidol lactate 0.1 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

* = Significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)

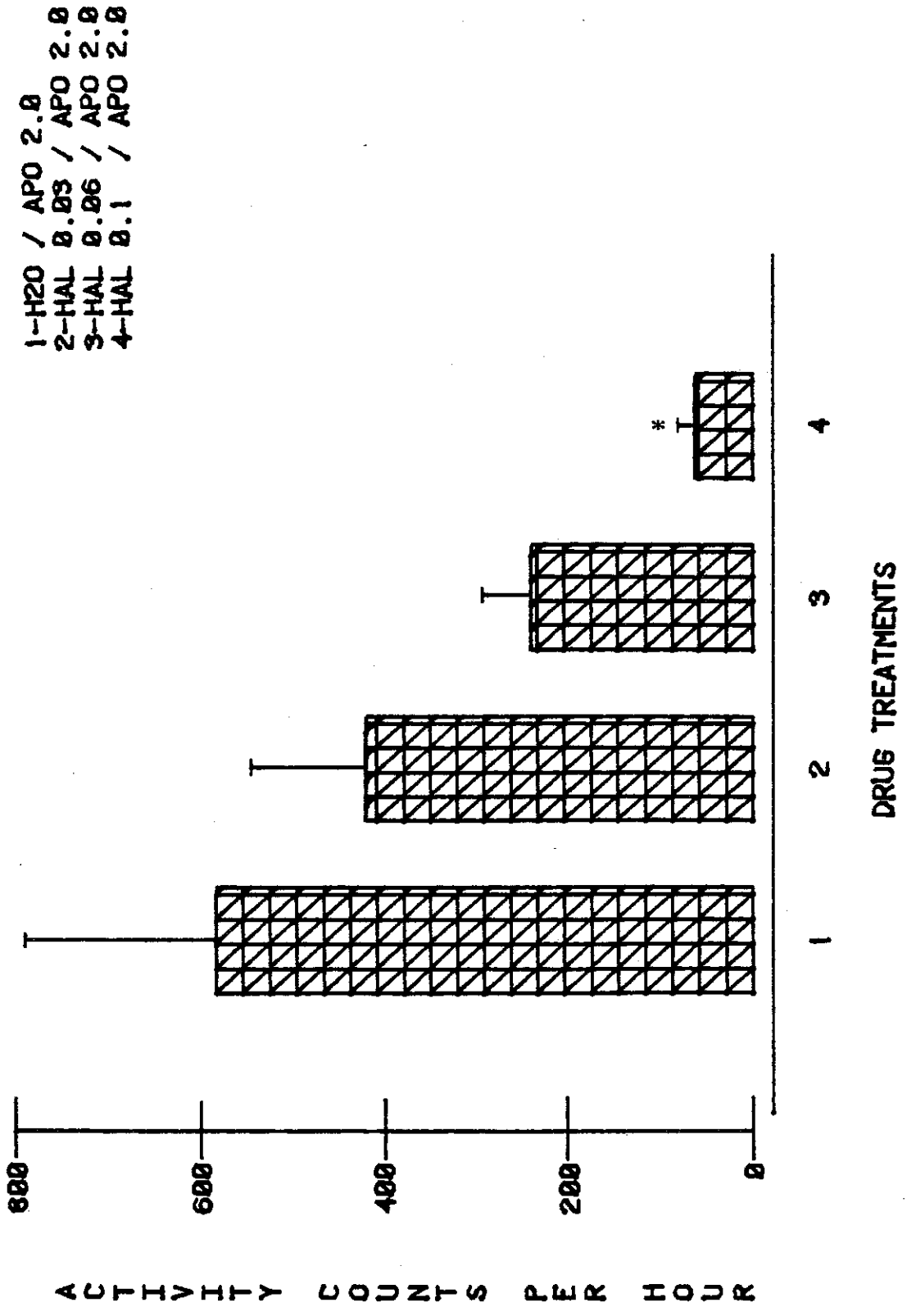

1-H2O / APO 2.0
2-HAL 0.03 / APO 2.0
3-HAL 0.06 / APO 2.0
4-HAL 0.1 / APO 2.0
ACTIVITY COUNTS PER HOUR
800
600
400
200
0
1
2
3
4
*
DRUG TREATMENTS

Figure 7. The mean effects of pretreatment (-30 minutes) with lenperone hydrochloride on the locomotor activity effects of intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

LEN 0.03/APO 2.0 = Lenperone HCl 0.03 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

LEN 0.1/APO 2.0 = Lenperone HCl 0.1 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

LEN 0.3/APO 2.0 = Lenperone HCl 0.3 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

* = Significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)

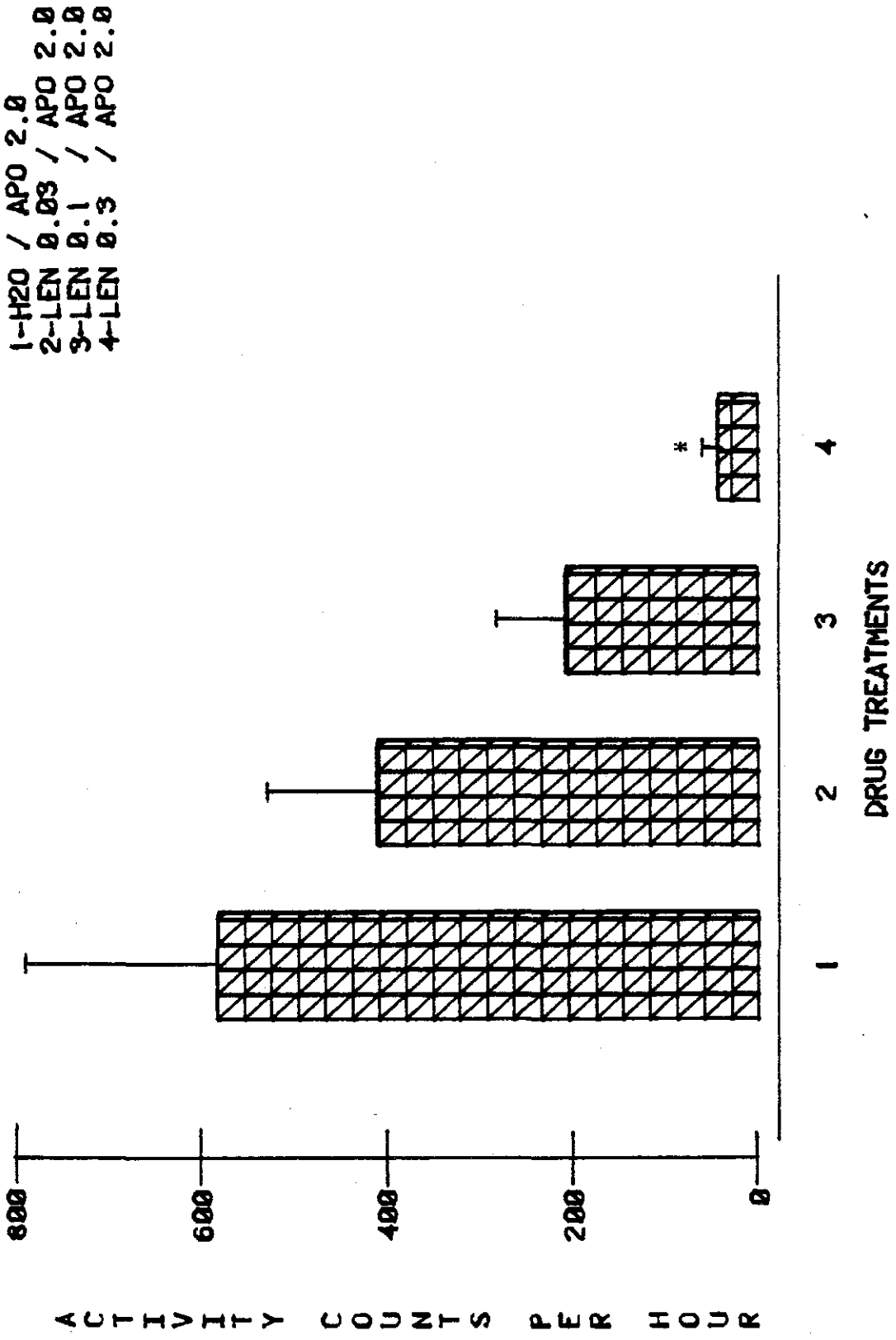

1-H20 / APO 2.0
2-LEN 0.03 / APO 2.0
3-LEN 0.1 / APO 2.0
4-LEN 0.3 / APO 2.0
ACTIVITY COUNTS PER HOUR
800
600
400
200
0
1
2
3
4
DRUG TREATMENTS
*

Figure 8. The mean effects of pretreatment (-30 minutes) with chlorpromazine hydrochloride on the locomotor activity effects of intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

CPZ 0.5/APO 2.0 = Chlorpromazine HCl 0.5 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

CPZ 1.0/APO 2.0 = Chlorpromazine HCl 1.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

CPZ 2.0/APO 2.0 = Chlorpromazine HCl 2.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

* = Significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)

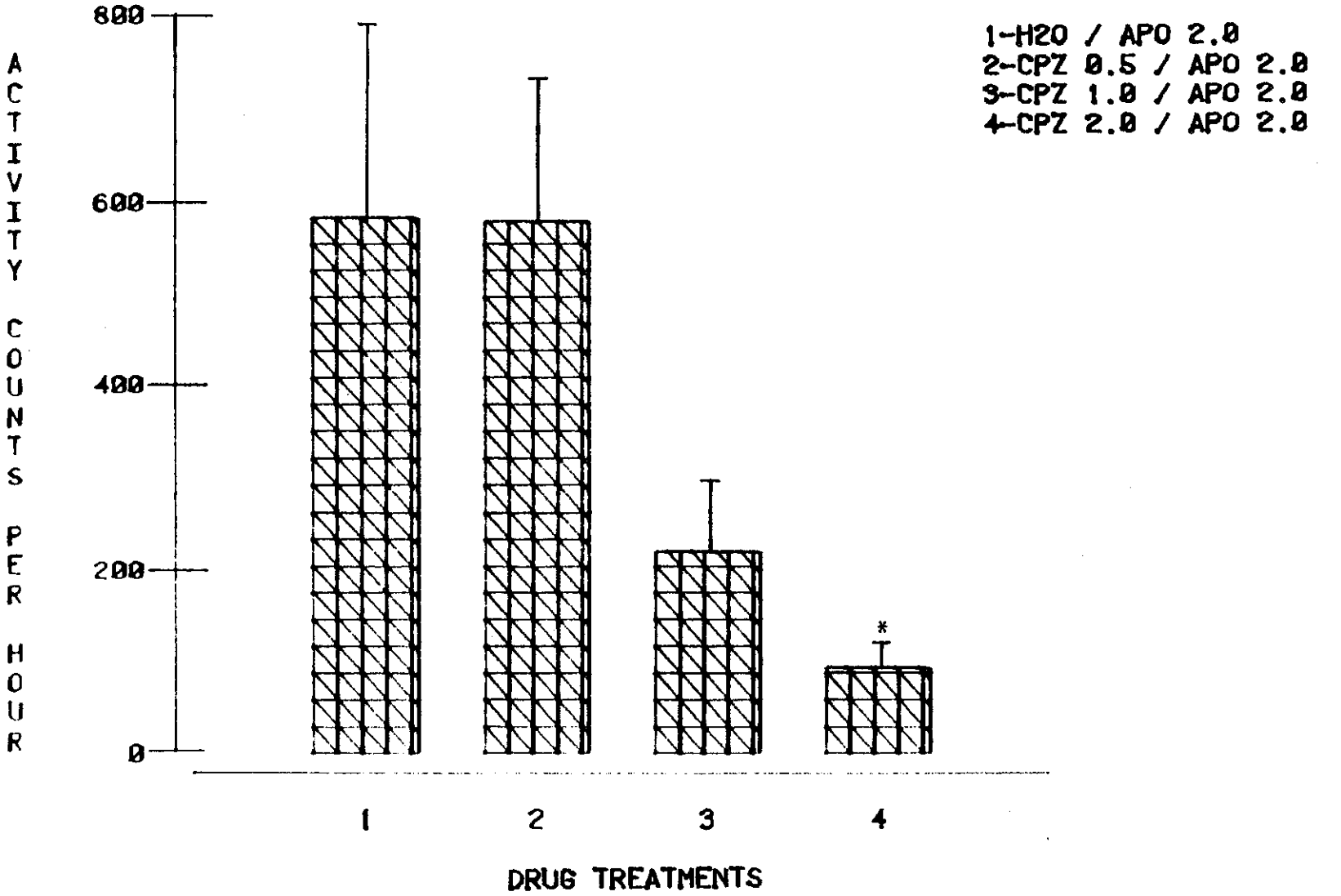

800
600
400
200
0
ACTIVITY COUNTS PER HOUR
1-H2O / APO 2.0
2-CPZ 0.5 / APO 2.0
3-CPZ 1.0 / APO 2.0
4-CPZ 2.0 / APO 2.0
*
1
2
3
4
DRUG TREATMENTS

Figure 9. The mean effects of pretreatment (-30 minutes) with thioridazine hydrochloride on the locomotor activity effects of intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

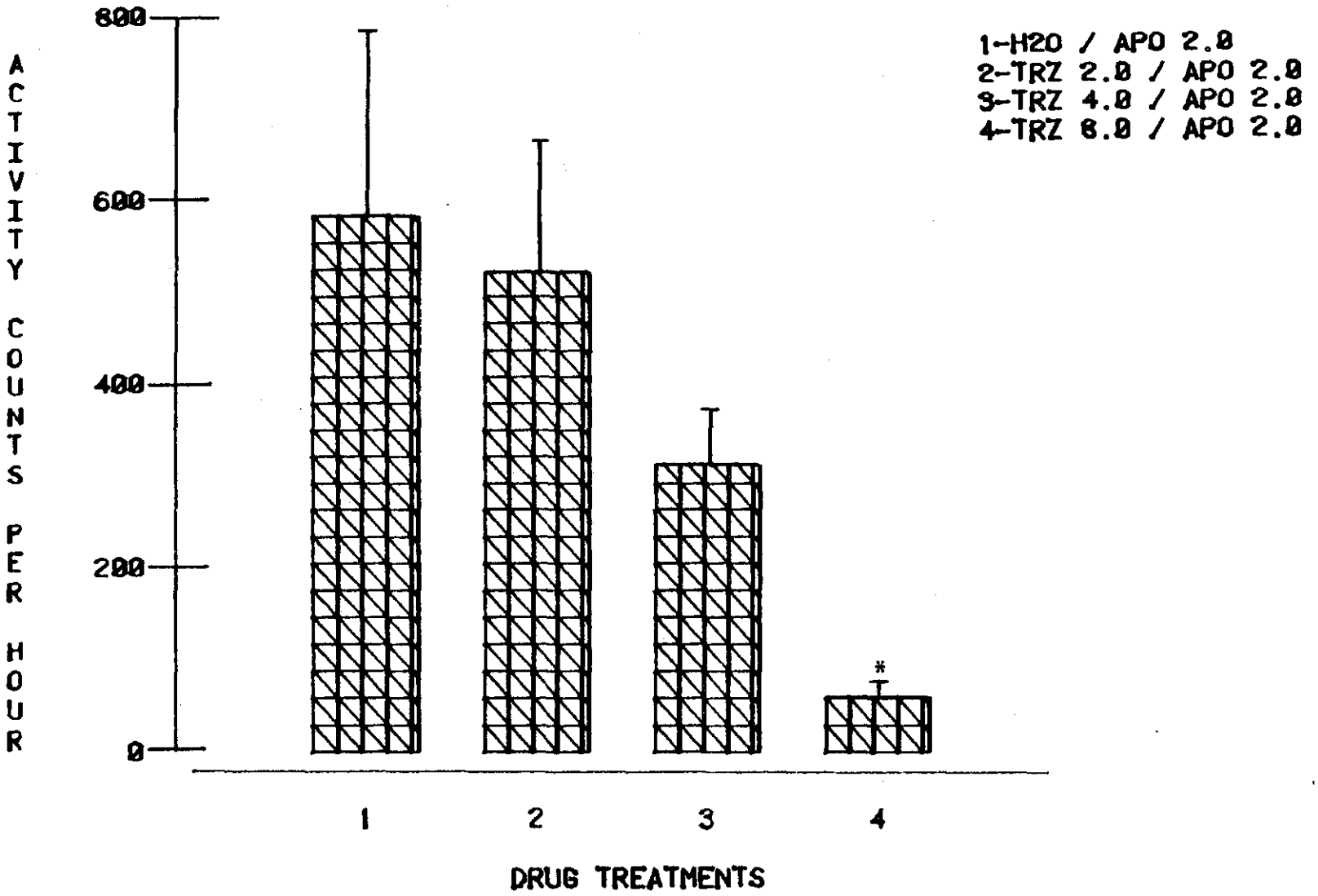
Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

TRZ 2.0/APO 2.0 = Thioridazine HCl 2.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

TRZ 4.0/APO 2.0 = Thioridazine HCl 4.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

TRZ 8.0/APO 2.0 = Thioridazine HCl 8.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

* = Significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)


800
600
400
200
0
ACTIVITY COUNTS PER HOUR
1-H2O / APO 2.0
2-TRZ 2.0 / APO 2.0
3-TRZ 4.0 / APO 2.0
4-TRZ 8.0 / APO 2.0
*
1
2
3
4
DRUG TREATMENTS

The behavior displayed then becomes progressively less intense until it becomes undistinguishable from that displayed by untreated animals approximately 60 minutes following treatment with apomorphine.

At all doses tested, HAL, LEN, CPZ and TRZ reduced the stereotypy score produced by APO at each time interval over the 90-minute testing period and reduced the total stereotypy score produced by APO in a dose-related manner (Figures 10, 11, 12, 13). The lowest dose of TRZ used to block APO-induced stereotypy was still higher than the highest doses of the other neuroleptics tested.

3. Catalepsy

The neuroleptic agents, HAL, LEN, CPZ and TRZ were all found to induce a dose-dependent cataleptic state when injected intraperitoneally into the rat. However, a cataleptic state was not induced in rats following intraperitoneal injection with double-distilled water. Catalepsy was induced in the rat with HAL at doses of 0.3 to 3.0 mg/kg, LEN at doses of 0.5 to 2.0 mg/kg, CPZ at doses of 4.0 to 9.0 mg/kg and TRZ at doses of 10.0 to 40.0 mg/kg. The highest dose of each neuroleptic tested induced a cataleptic state which was highly significantly different from vehicle-injected rats at all time intervals tested (Figures 14, 15, 16, 17). A cataleptic state was not induced by TRZ at doses less than 10 mg/kg. This dose of TRZ was still higher than the highest

Table III. The effect of pretreatment (-30 minutes) with either haloperidol lactate, lenperone hydrochloride, chlorpromazine hydrochloride or thioridazine hydrochloride on stereotyped behavior produced by intraperitoneal apomorphine hydrochloride over a 90-minute period in male rats.

Drug	Dose	N	Total Stereotyped Behavior Score	% of Maximum Inhibition of Stereotyped Behavior
Apomorphine	2.0	9	55.78 ± 0.83	
Haloperidol	0.03	6	40.0 ± 1.59	28.29 ± 2.85
	0.06	6	31.0 ± 2.02	44.42 ± 3.62
	0.1	6	19.83 ± 0.48	64.44 ± 0.86
Lenperone	0.03	6	44.83 ± 1.54	16.64 ± 1.37
	0.1	6	29.33 ± 2.43	47.41 ± 4.36
	0.3	6	21.17 ± 1.30	62.05 ± 2.33
Chlorpromazine	0.5	7	46.71 ± 2.02	16.25 ± 3.62
	1.0	9	34.11 ± 2.24	38.85 ± 4.01
	2.0	8	22.63 ± 1.36	59.44 ± 2.44
Thioridazine	9.0	9	35.11 ± 1.65	37.05 ± 2.95
	12.0	7	26.14 ± 0.83	53.13 ± 1.49
	16.0	7	20.57 ± 0.75	63.12 ± 1.35

Dose is expressed as mg/kg. Values are reported as the mean ± 1.0 SEM.
N represents the number of animals used.

Figure 10. The mean effects of pretreatment (-30 minutes) with haloperidol lactate on stereotyped behavior produced by intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HC1 2.0 mg/kg (N=9)

HAL .03/APO 2.0 = Haloperidol lactate 0.03 mg/kg followed by apomorphine HC1 2.0 mg/kg (N=6)

HAL .06/APO 2.0 = Haloperidol lactate 0.06 mg/kg followed by apomorphine HC1 2.0 mg/kg (N=6)

HAL 0.1/APO 2.0 = Haloperidol lactate 0.1 mg/kg followed by apomorphine HC1 2.0 mg/kg (N=6)

* = Significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)

** = Highly significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.01$)

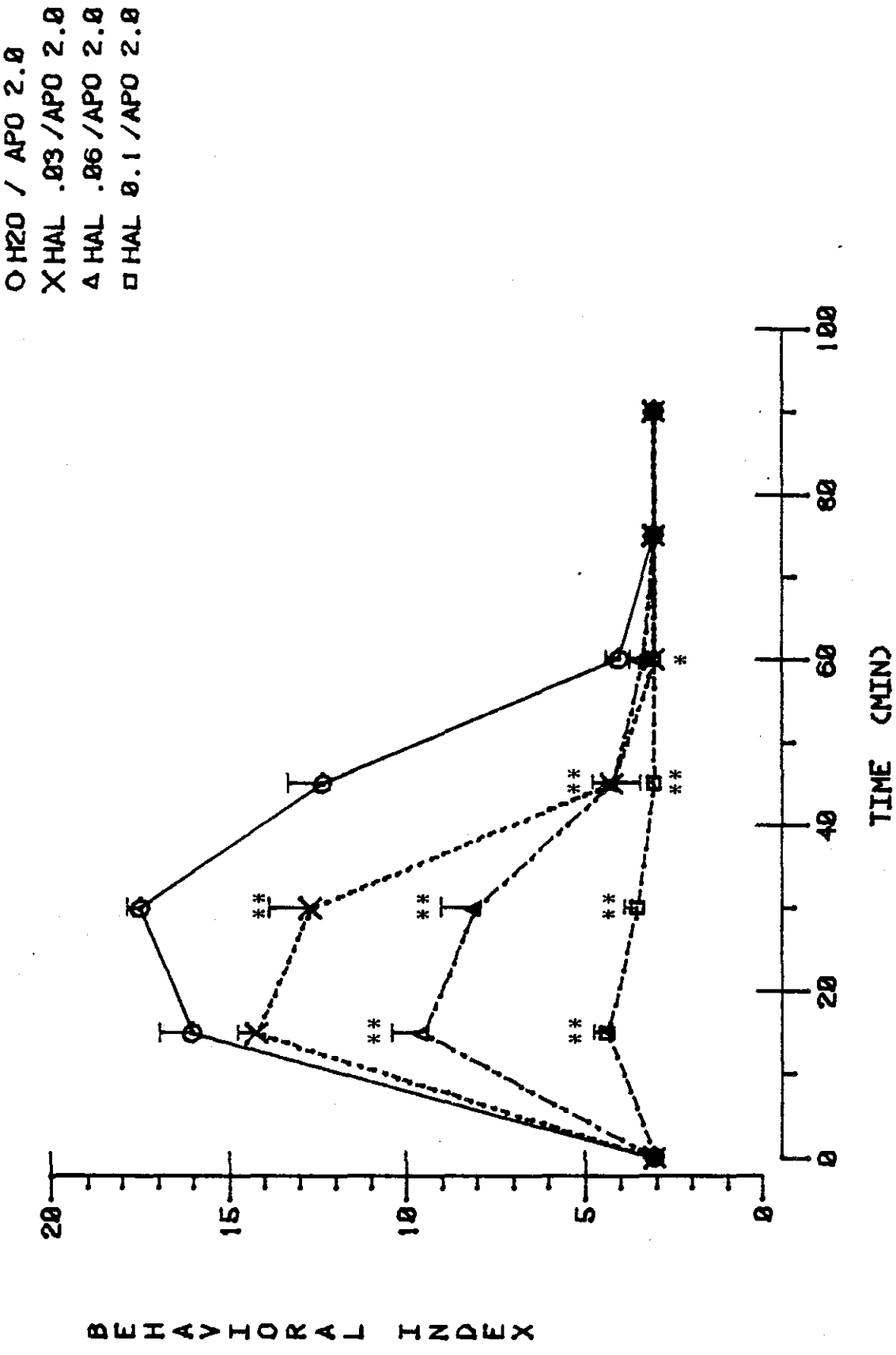

O H2O / APO 2.0
X HAL .03 /APO 2.0
Δ HAL .06 /APO 2.0
□ HAL 0.1 /APO 2.0
BEHAVIORAL INDEX
20
15
10
5
0
0
20
40
60
80
100
TIME (MIN)
*
**

Figure 11. The mean effects of pretreatment (-30 minutes) with lenperone hydrochloride on stereotyped behavior produced by intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

LEN .03/APO 2.0 = Lenperone HCl 0.03 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

LEN .01/APO 2.0 = Lenperone HCl 0.1 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

LEN 0.3/APO 2.0 = Lenperone HCl 0.3 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

* = Significantly different from rats injected with H20/APO 2.0 ($P < 0.05$)

** = Highly significantly different from rats injected with H20/APO 2.0 ($P < 0.01$)

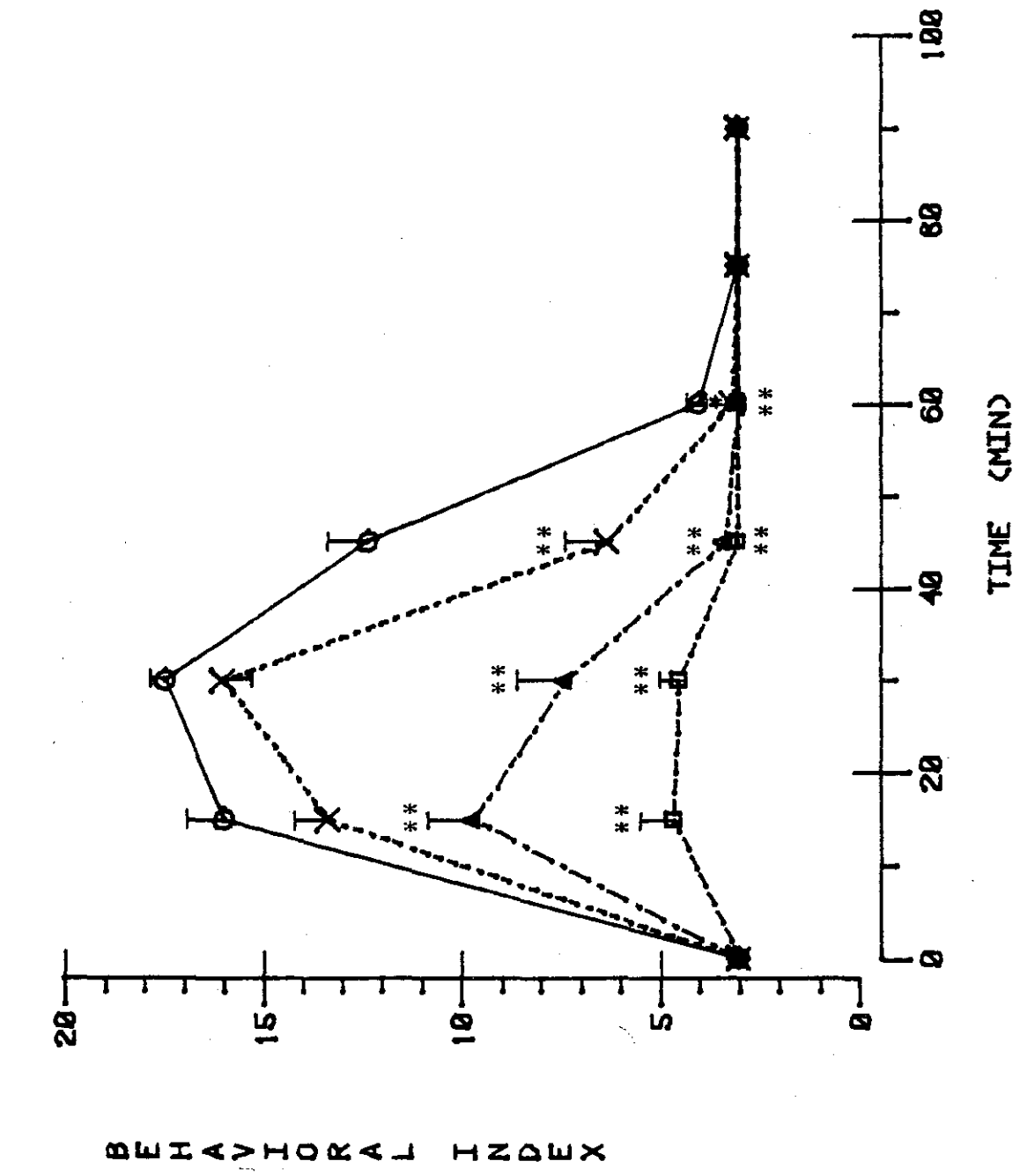

O H2O / APO 2.0
X LEN .03 /APO 2.0
Δ LEN 0.1 /APO 2.0
□ LEN 0.3 /APO 2.0
BEHAVIORAL INDEX
20
15
10
5
0
0
20
40
60
80
100
TIME (MIN)
**

Figure 12. The mean effects of pretreatment (-30 minutes) with chlorpromazine hydrochloride on stereotyped behavior produced by intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

CPZ 0.5/APO 2.0 = Chlorpromazine HCl 0.5 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=7)

CPZ 1.0/APO 2.0 = Chlorpromazine HCl 1.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

CPZ 2.0/APO 2.0 = Chlorpromazine HCl 2.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=8)

** = Highly significantly different from rats injected with H20/APO 2.0 ($P < 0.01$)

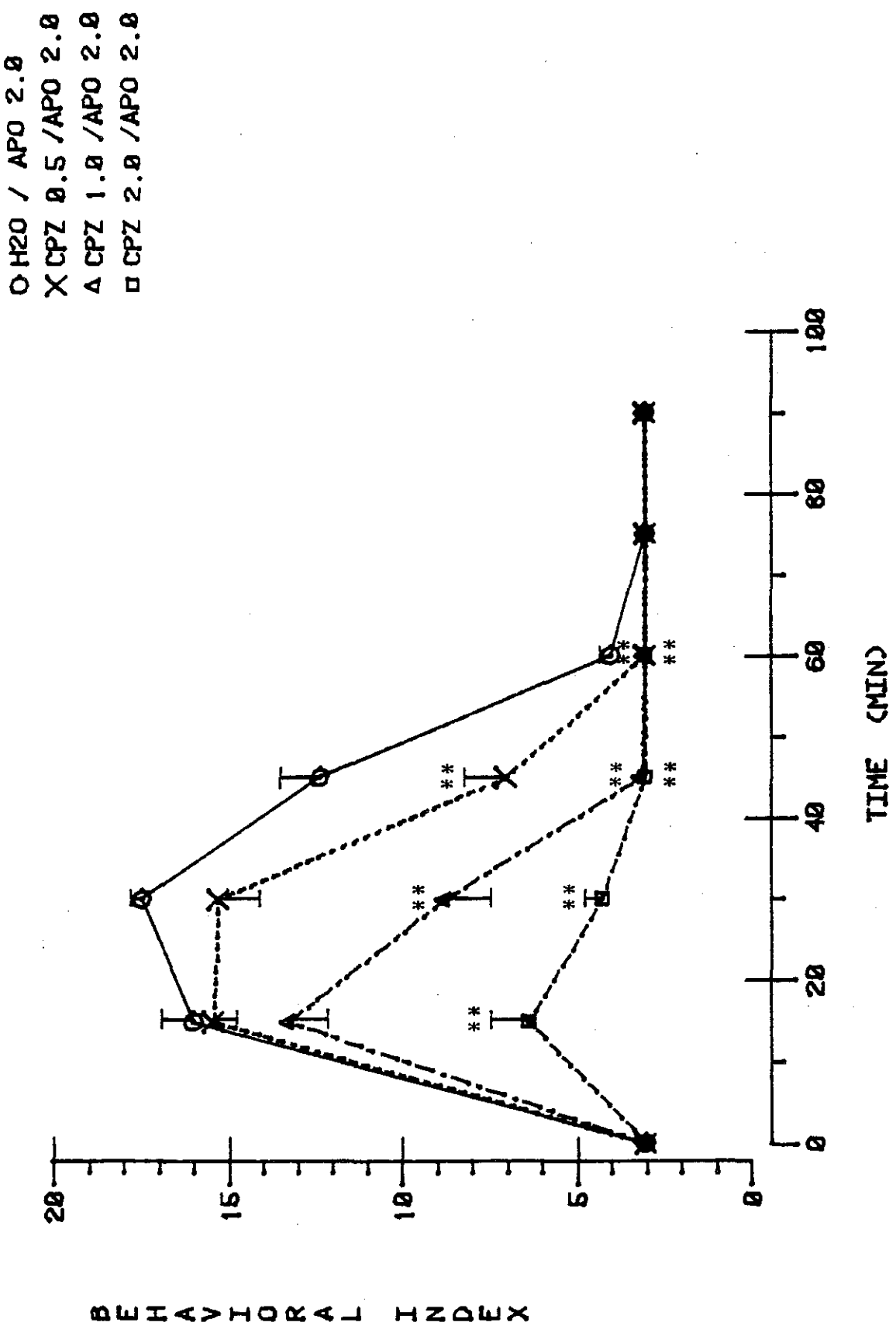

O H2O / APO 2.0
X CPZ 0.5 /APO 2.0
Δ CPZ 1.0 /APO 2.0
□ CPZ 2.0 /APO 2.0
BEHAVHORAL INDEX
20
15
10
5
0
0
20
40
60
80
100
TIME (MIN)
**

Figure 13. The mean effects of pretreatment (-30 minutes) with thioridazine hydrochloride on stereotyped behavior produced by intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

TRZ 9/APO 2.0 = Thioridazine HCl 9.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

TRZ 12/APO 2.0 = Thioridazine HCl 12.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=7)

TRZ 16/APO 2.0 = Thioridazine HCl 16.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=7)

* = Significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)

** = Highly significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.01$)

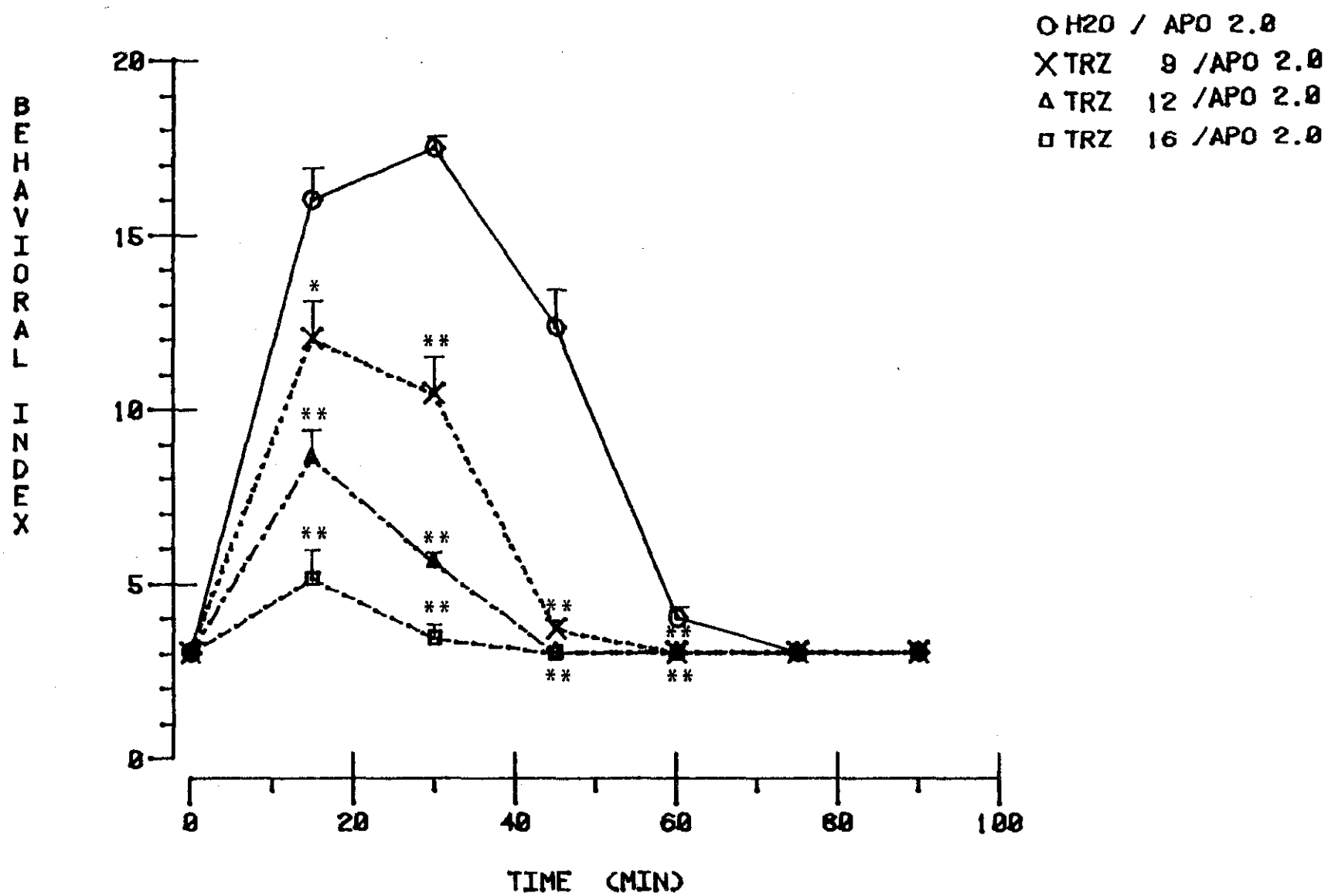

O H2O / APO 2.0
X TRZ 9 /APO 2.0
Δ TRZ 12 /APO 2.0
□ TRZ 16 /APO 2.0
BEHAVIORAL INDEX
20
15
10
5
0
0
20
40
60
80
100
TIME (MIN)

Table IV. The effect of treatment with either haloperidol lactate, lenperone hydrochloride, chlorpromazine hydrochloride or thioridazine hydrochloride on the ability of male rats to maintain a cataleptic position over a 90-minute testing period.

Drug	Dose	N	Total Bar Time	% of Maximum Bar Time
Haloperidol	0.3	7	120.57 ± 21.25	16.75 ± 2.95
	1.0	7	377.71 ± 38.17	52.46 ± 5.30
	3.0	7	654.14 ± 15.66	90.85 ± 2.18
Lenperone	0.5	8	91.38 ± 14.32	12.69 ± 1.99
	1.0	9	305.33 ± 56.51	42.41 ± 7.85
	2.0	8	588.88 ± 25.33	81.79 ± 3.52
Chlorpromazine	4.0	8	72.25 ± 10.09	10.03 ± 1.40
	6.0	6	346.33 ± 22.77	48.10 ± 3.16
	9.0	6	598.00 ± 32.42	83.06 ± 4.50
Thioridazine	10.0	7	69.57 ± 8.04	9.66 ± 1.12
	20.0	8	314.25 ± 21.41	43.64 ± 2.97
	40.0	8	541.12 ± 12.28	75.16 ± 1.70

Dose is expressed as mg/kg. Values are reported as the mean ± 1.0 SEM. N represents the number of animals used. Total bar time is expressed in seconds.

Figure 14. The mean effects of treatment with haloperidol lactate on the ability of male rats to maintain a cataleptic position (vertical bars indicate 1.0 SEM)

Key: H20 = Double-distilled water 1.0 ml/kg (N=6)

HAL 0.3 = Haloperidol lactate 0.3 mg/kg (N=7)

HAL 1.0 = Haloperidol lactate 1.0 mg/kg (N=7)

HAL 3.0 = Haloperidol lactate 3.0 mg/kg (N=7)

* = Significantly different from rats injected with H20 ($P < 0.05$)

** = Highly significantly different from rats injected with H20 ($\underline{P} < 0.01$)

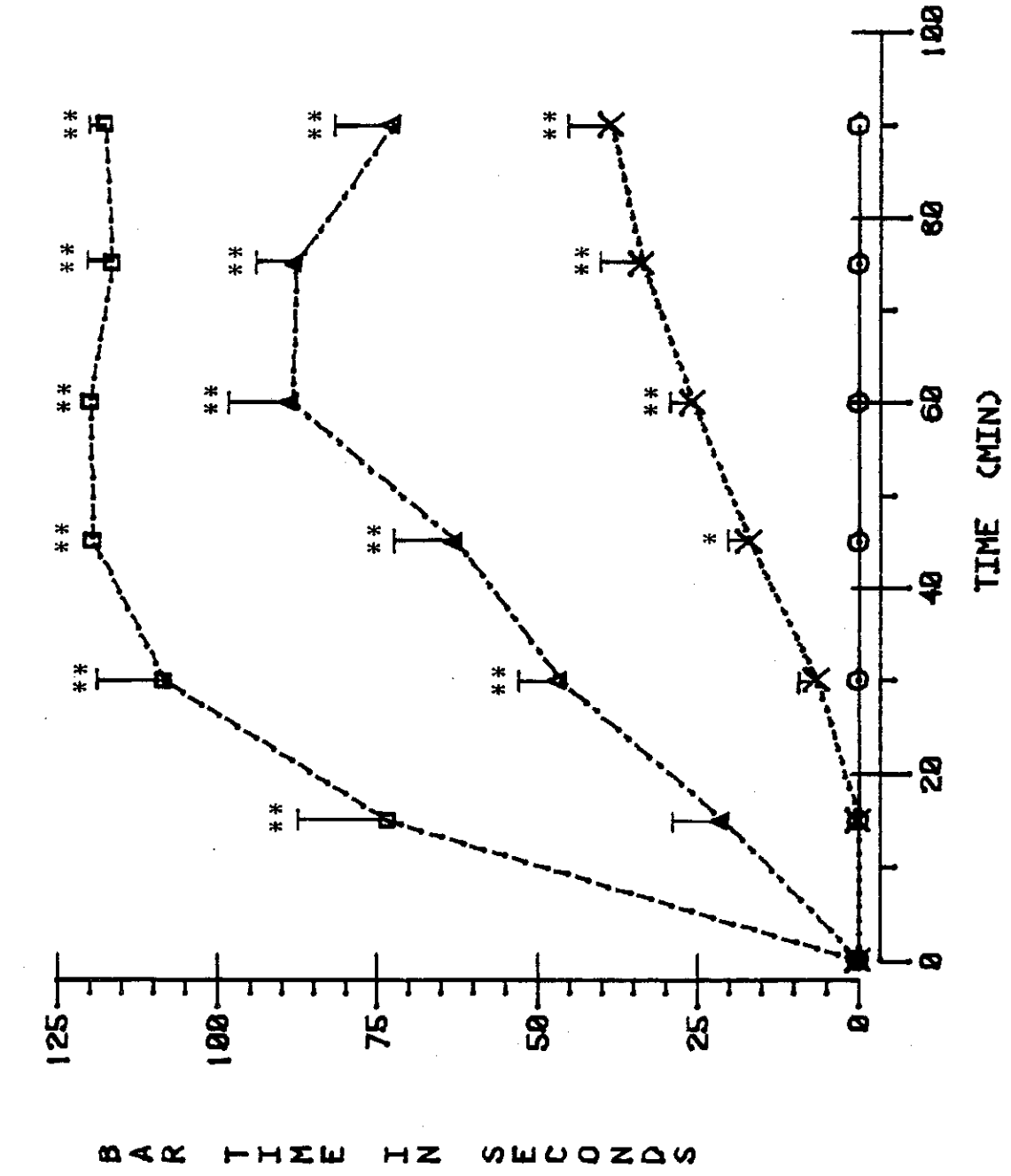

O H2O
X HAL 0.3
Δ HAL 1.0
□ HAL 3.0
BAR TIME IN SECONDS
125
100
75
50
25
0
0
20
40
60
80
100
TIME (MIN)

Figure 15. The mean effects of treatment with lenperone hydrochloride on the ability of male rats to maintain a cataleptic position (vertical bars indicate 1.0 SEM).

Key: H20 = Double-distilled water 1.0 ml/kg (N=6)

LEN 0.5 = Lenperone HCl 0.5 mg/kg (N=8)

LEN 1.0 = Lenperone HCl 1.0 mg/kg (N=9)

LEN 2.0 = Lenperone HCl 2.0 mg/kg (N=8)

* = Significantly different from rats injected with H20 ($\underline{P} < 0.05$)

** = Highly significantly different from rats injected with H20 ($\underline{P} < 0.01$)

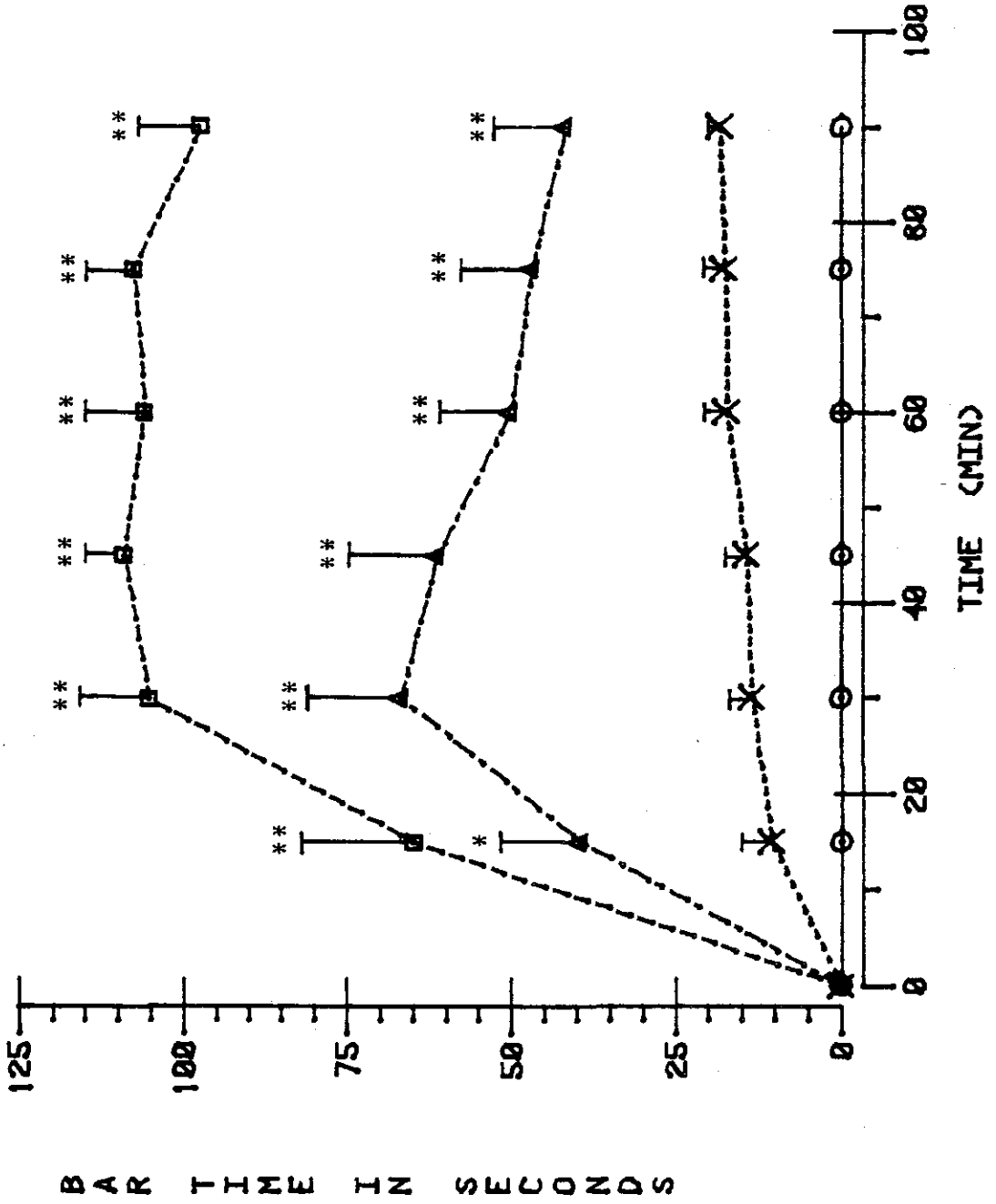

O H2O
X LEN 0.5
Δ LEN 1.0
□ LEN 2.0
BAR TIME IN SECONDS
125
100
75
50
25
0
0
20
40
60
80
100
TIME (MIN)

Figure 16. The mean effects of treatment with chlorpromazine hydrochloride on the ability of male rats to maintain a cataleptic position (vertical bars indicate 1.0 SEM).

Key: H20 = Double-distilled water 1.0 ml/kg (N=6)

CPZ 4.0 = Chlorpromazine HCl 4.0 mg/kg (N=8)

CPZ 6.0 = Chlorpromazine HCl 6.0 mg/kg (N=6)

CPZ 9.0 = Chlorpromazine HCl 9.0 mg/kg (N=6)

* = Significantly different from rats injected with H20 ($\underline{P} < 0.05$)

** = Highly significantly different from rats injected with H20 ($\underline{P} < 0.01$)

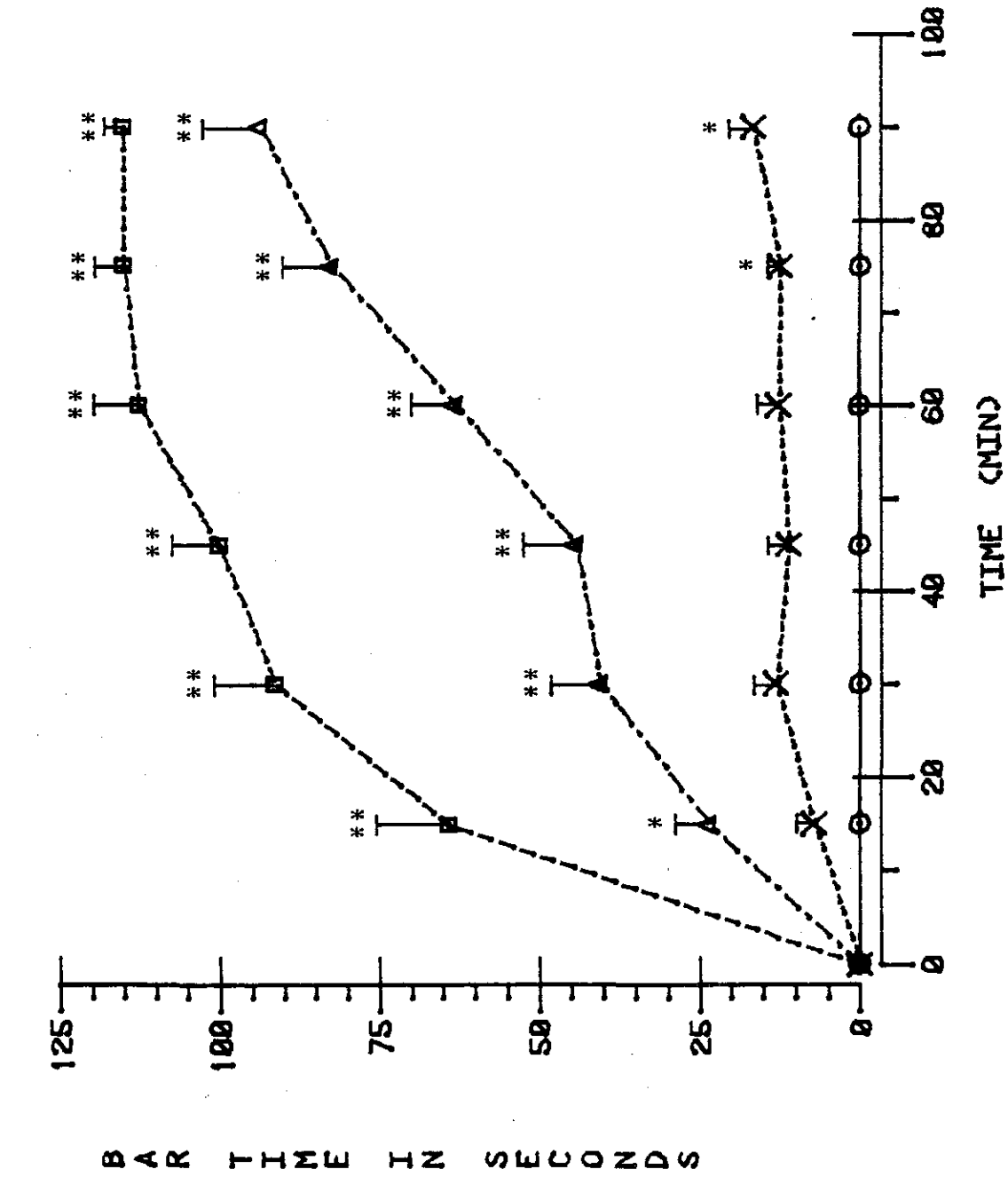

O H2O
X CPZ 4.0
Δ CPZ 6.0
□ CPZ 9.0
BAR TIME IN SECONDS
125
100
75
50
25
0
0
20
40
60
80
100
TIME (MIN)

Figure 17. The mean effects of treatment with thioridazine hydrochloride on the ability of male rats to maintain a cataleptic position (vertical bars indicate 1.0 SEM).

Key: H20 = Double-distilled water 1.0 ml/kg (N=6)

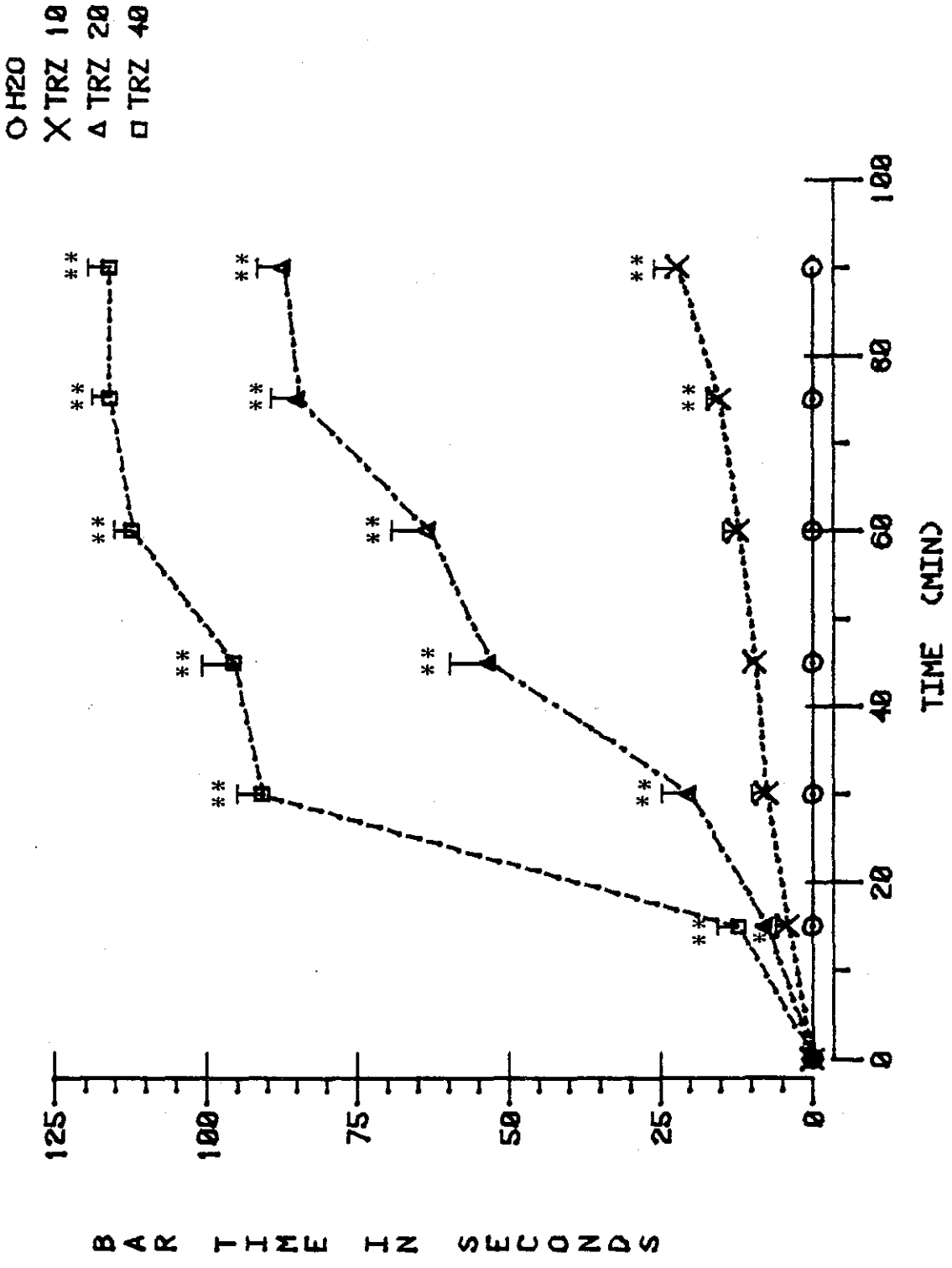
TRZ 10 = Thioridazine HCl 10.0 mg/kg (N=7)

TRZ 20 = Thioridazine HCl 20.0 mg/kg (N=8)

TRZ 40 = Thioridazine HCl 40.0 mg/kg (N=8)

* = Significantly different from rats injected with H20 ($\underline{P} < 0.05$)

** = Highly significantly different from rats injected with H20 ($\underline{P} < 0.01$)


O H2O
X TRZ 10
Δ TRZ 20
□ TRZ 40
BAR TIME IN SECONDS
125
100
75
50
25
0
0
20
40
60
80
100
TIME (MIN)

doses of the other neuroleptics tested.

4. Body Temperature

APO 2.0 mg/kg produced a dose-related hypothermia when injected intraperitoneally into rats. The maximum drop in body temperature, $-1.08^{\circ} \pm 0.12^{\circ}C$ (mean $\pm$ SEM), occurred 15 minutes following the injection of APO. Pretreatment with HAL at 0.1 and 0.3 mg/kg highly significantly antagonized the maximum drop in body temperature induced by APO 2.0 mg/kg (Figure 19). Pretreatment with HAL 0.03 mg/kg potentiated the hypothermic action of APO between 30 and 90 minutes following the APO injection (Figure 19). When tested alone, intraperitoneal HAL 0.3 mg/kg did not significantly affect body temperature in the rat (Figure 18). Pretreatment with LEN at 0.3 and 1.0 mg/kg highly significantly antagonized the maximum drop in body temperature induced by APO 2.0 mg/kg (Figure 20). Pretreatment with LEN 0.1 mg/kg potentiated the hypothermic action of APO between 30 and 90 minutes following the APO injection to a degree that was not significant (Figure 20). When tested alone, intraperitoneal LEN 1.0 mg/kg did not significantly affect body temperature in the rat (Figure 18). Pretreatment with CPZ at 1.7 and 3.0 mg/kg highly significantly antagonized the maximum drop in body temperature induced by APO 2.0 mg/kg (Figure 21). Pretreatment with CPZ 1.0 mg/kg potentiated the hypothermic action of APO between 30 and 90 minutes following the APO injection to a significant degree

Table V. The effect of pretreatment (-30 minutes) with either haloperidol lactate, lenperone hydrochloride, chlorpromazine hydrochloride or thioridazine hydrochloride on the rectal temperature effects of intraperitoneal apomorphine hydrochloride over a 90-minute testing period in male rats.

Drug	Dose	N	Maximum Drop in Body Temperature	% of Maximum Inhibition of Hypothermia
Apomorphine	2.0	9	-1.08 ± 0.12	
Haloperidol	0.03	6	-1.03 ± 0.08	4.32 ± 7.81
	0.1	6	-0.50 ± 0.15	53.7 ± 13.94
	0.3	6	0	100 ± 0
Lenperone	0.1	6	-0.97 ± 0.15	10.49 ± 14.28
	0.3	6	-0.30 ± 0.11	72.22 ± 10.14
	1.0	6	0	100 ± 12.87
Chlorpromazine	1.0	8	-1.14 ± 0.09	-5.32 ± 8.38
	1.7	6	-0.73 ± 0.13	32.10 ± 12.11
	3.0	9	-0.39 ± 0.09	63.99 ± 7.94
Thioridazine	1.0	6	-1.12 ± 0.13	-3.40 ± 12.05
	1.7	6	-0.72 ± 0.09	33.64 ± 8.76
	3.0	6	-0.30 ± 0.10	72.22 ± 9.56

Dose is expressed as mg/kg. Values are reported as the mean + 1.0 SEM.
N represents the number of animals used.

Figure 18. The mean effects of intraperitoneal haloperidol lactate, lenperone hydrochloride, chlorpromazine hydrochloride or thioridazine hydrochloride on the rectal temperature of male rats (vertical bars indicate 1.0 SEM).

Key: H2O = Double-distilled water 1.0 ml/kg (N=6)

HAL 0.3 = Haloperidol lactate 0.3 mg/kg (N=6)

LEN 1.0 = Lenperone HCl 1.0 mg/kg (N=6)

CPZ 3.0 = Chlorpromazine HCl 3.0 mg/kg (N=6)

TRZ 3.0 = Thioridazine HCl 3.0 mg/kg (N=6)

* = Significantly different from rats injected with H2O ($\underline{P} < 0.05$)

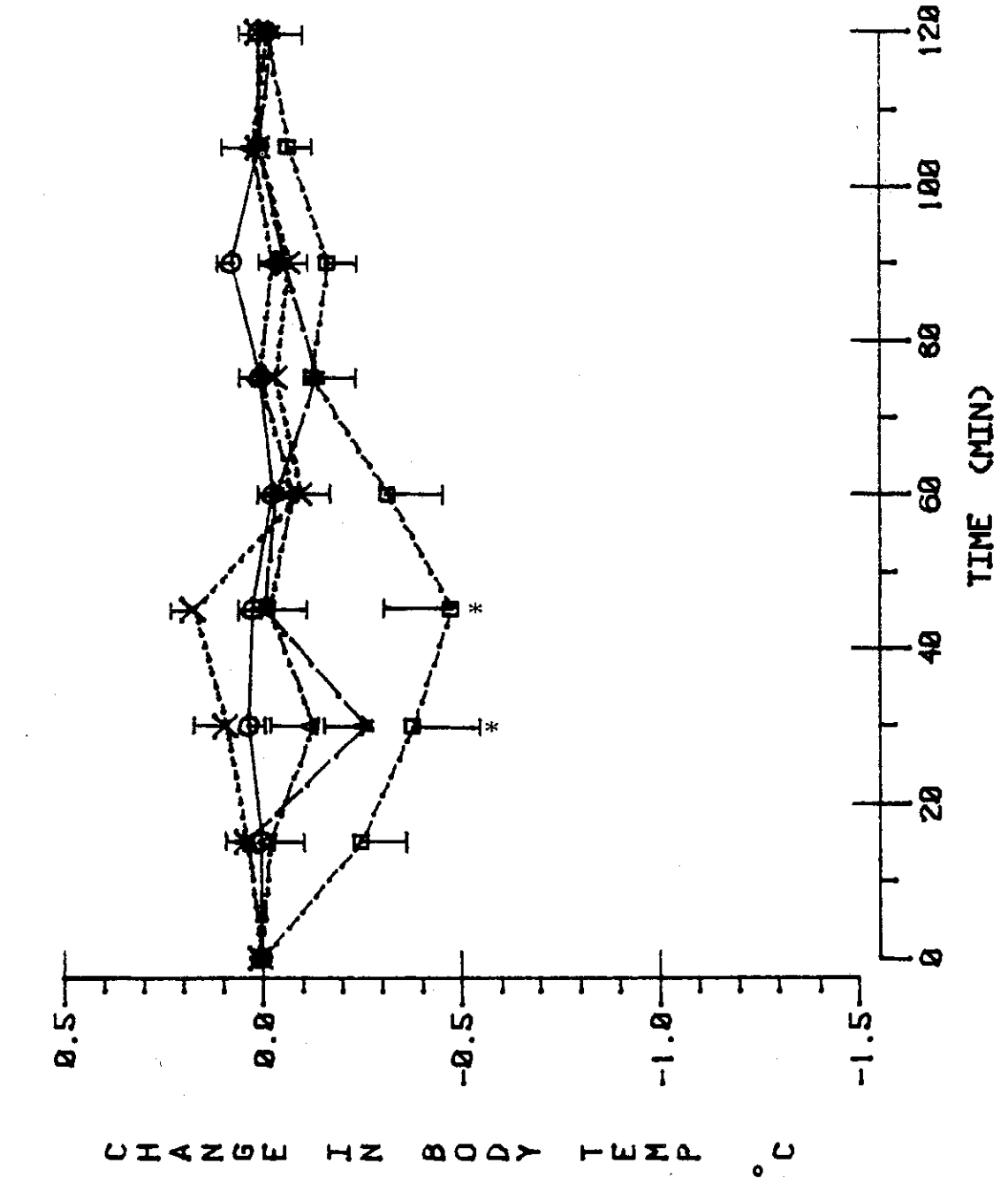

O H2O
X HAL 0.3
Δ LEN 1.0
□ CPZ 3.0
★ TRZ 3.0
0.5
0.0
-0.5
-1.0
-1.5
CHANGE IN BODY TEMP °C
0
20
40
60
80
100
120
TIME (MIN)
*
*

Figure 19. The mean effects of pretreatment (-30 minutes) with haloperidol lactate on the body temperature effects of intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

HAL .03/APO 2.0 = Haloperidol lactate 0.03 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

HAL 0.1/APO 2.0 = Haloperidol lactate 0.1 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

HAL 0.3/APO 2.0 = Haloperidol lactate 0.3 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

* = Significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)

** = Highly significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.01$)

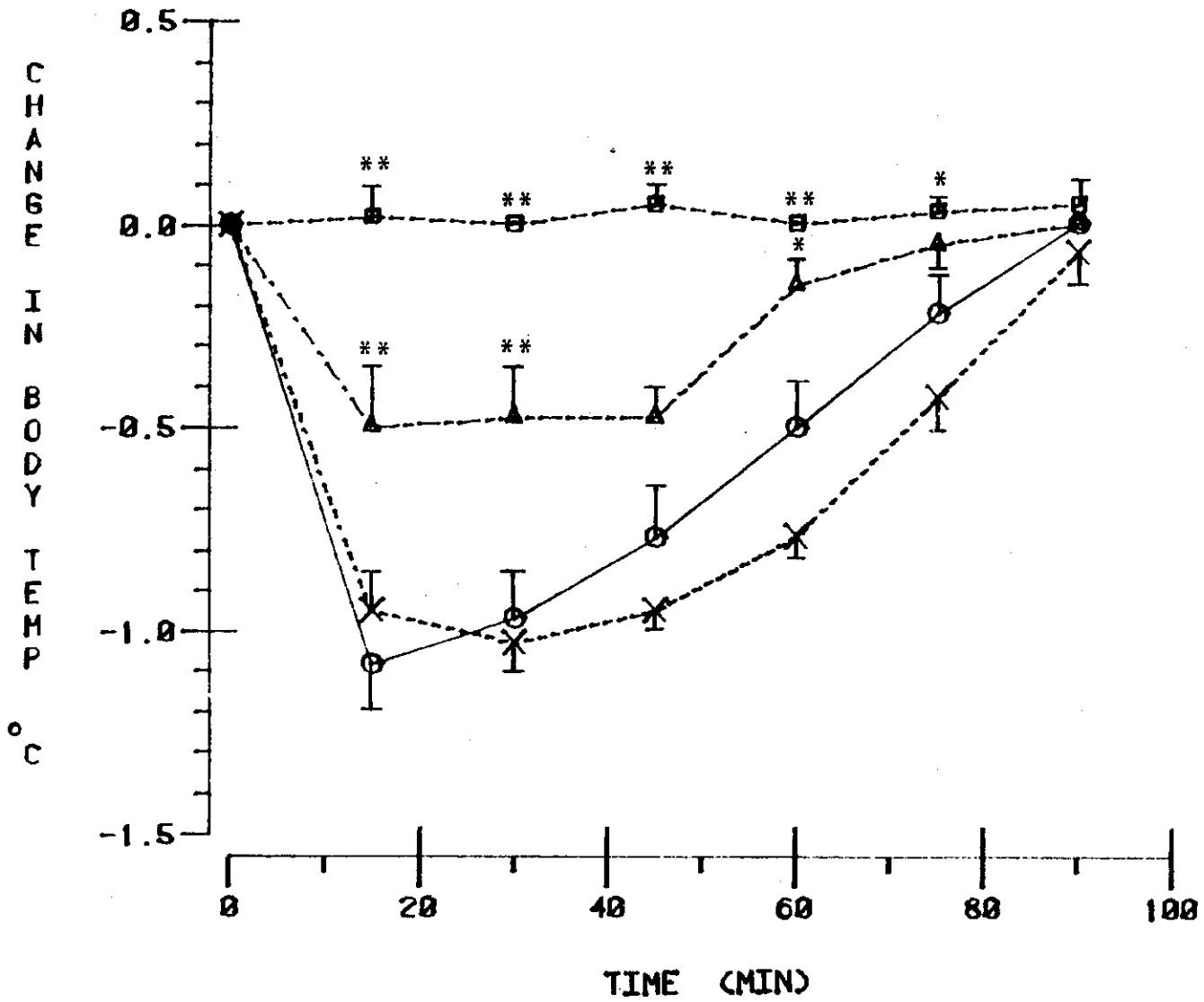

O H2O / APO 2.0
X HAL .03 /APO 2.0
Δ HAL 0.1 /APO 2.0
□ HAL 0.3 /APO 2.0
CHANGE IN BODY TEMP °C
0.5
0.0
-0.5
-1.0
-1.5
0
20
40
60
80
100
TIME (MIN)
**
**
**
**
*
**
**
*

Figure 20. The mean effects of pretreatment (-30 minutes) with lenperone hydrochloride on the body temperature effects of intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

LEN 0.1/APO 2.0 = Lenperone HCl 0.1 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

LEN 0.3/APO 2.0 = Lenperone HCl 0.3 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

LEN 1.0/APO 2.0 = Lenperone HCl 1.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

* = Significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)

** = Highly significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.01$)

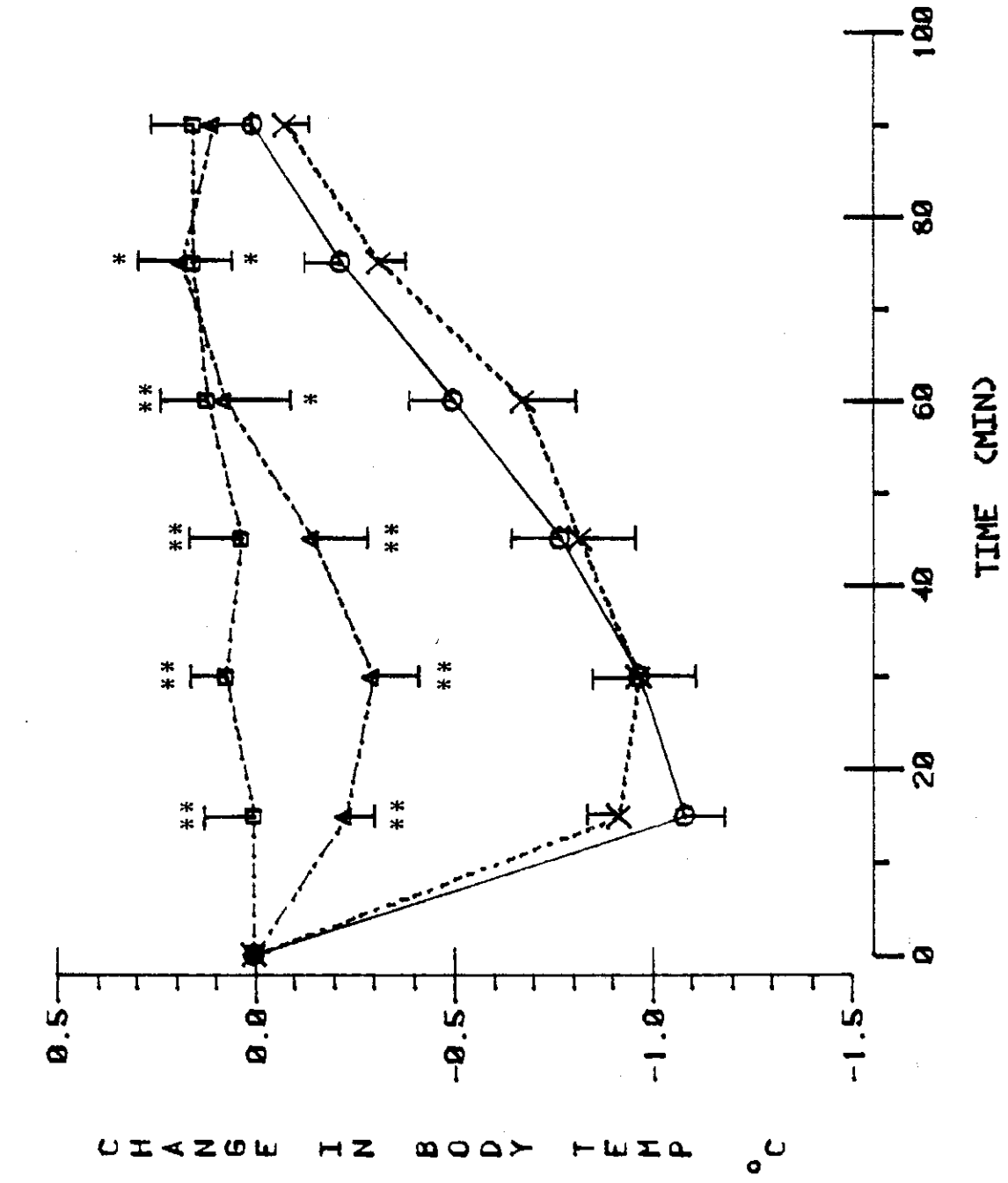

○ H2O / APO 2.0
× LEN 0.1 /APO 2.0
△ LEN 0.3 /APO 2.0
□ LEN 1.0 /APO 2.0
CHANGE IN BODY TEMP °C
0.5
0.0
-0.5
-1.0
-1.5
0
20
40
60
80
100
TIME (MIN)

Figure 21. The mean effects of pretreatment (-30 minutes) with chlorpromazine hydrochloride on the body temperature effects of intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM)

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

CPZ 1.0/APO 2.0 = Chlorpromazine HCl 1.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=8)

CPZ 1.7/APO 2.0 = Chlorpromazine HCl 1.7 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

CPZ 3.0/APO 2.0 = Chlorpromazine HCl 3.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

* = Significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)

** = Highly significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.01$)

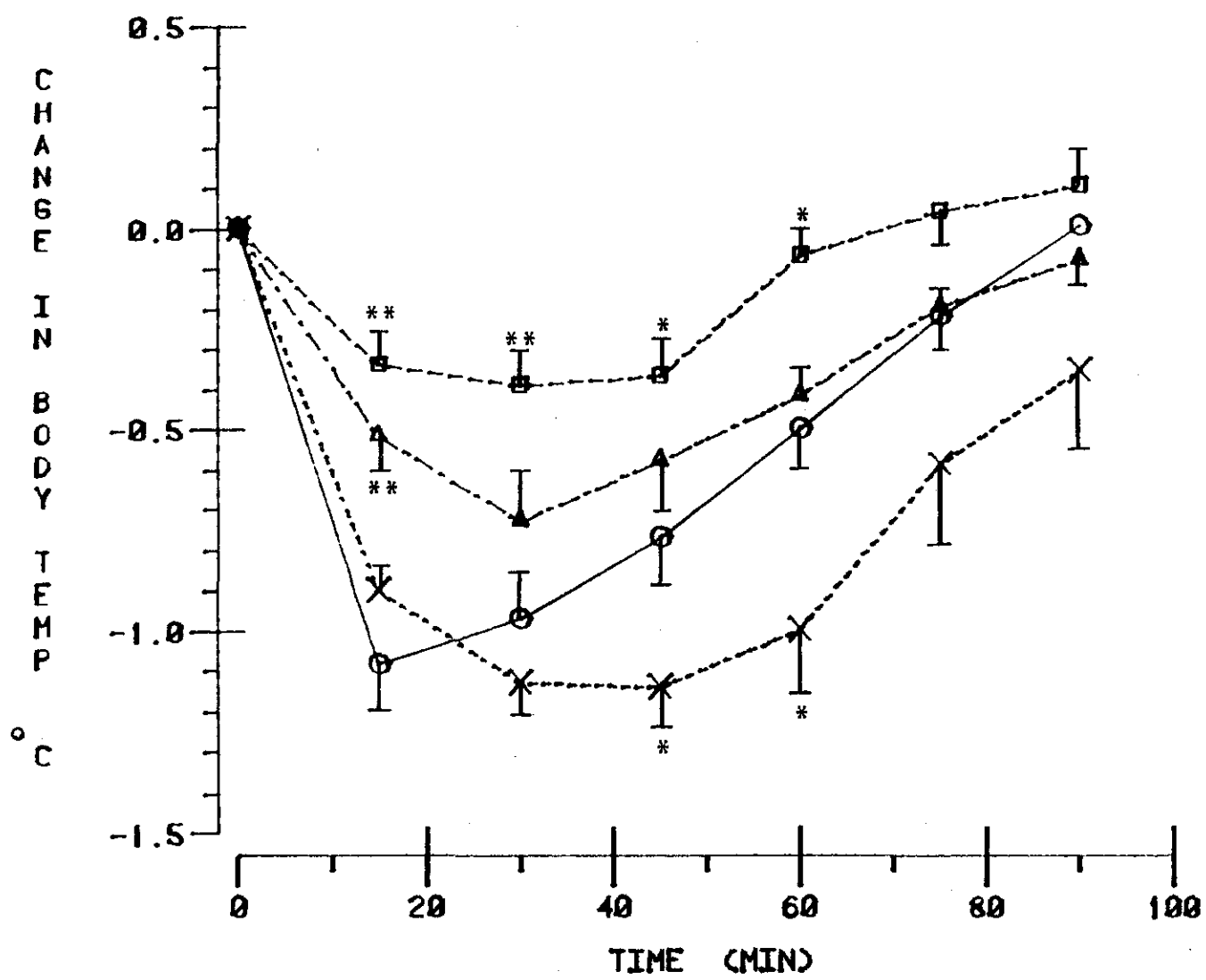

H2O / APO 2.0
CPZ 1.0 /APO 2.0
CPZ 1.7 /APO 2.0
CPZ 3.0 /APO 2.0
CHANGE IN BODY TEMP °C
0.5
0.0
-0.5
-1.0
-1.5
0
20
40
60
80
100
TIME (MIN)

Figure 22. The mean effects of pretreatment (-30 minutes) with thioridazine hydrochloride on the body temperature effects of intraperitoneal apomorphine hydrochloride administered to male rats (vertical bars indicate 1.0 SEM).

Key: H20/APO 2.0 = Double-distilled water 1.0 ml/kg followed by apomorphine HCl 2.0 mg/kg (N=9)

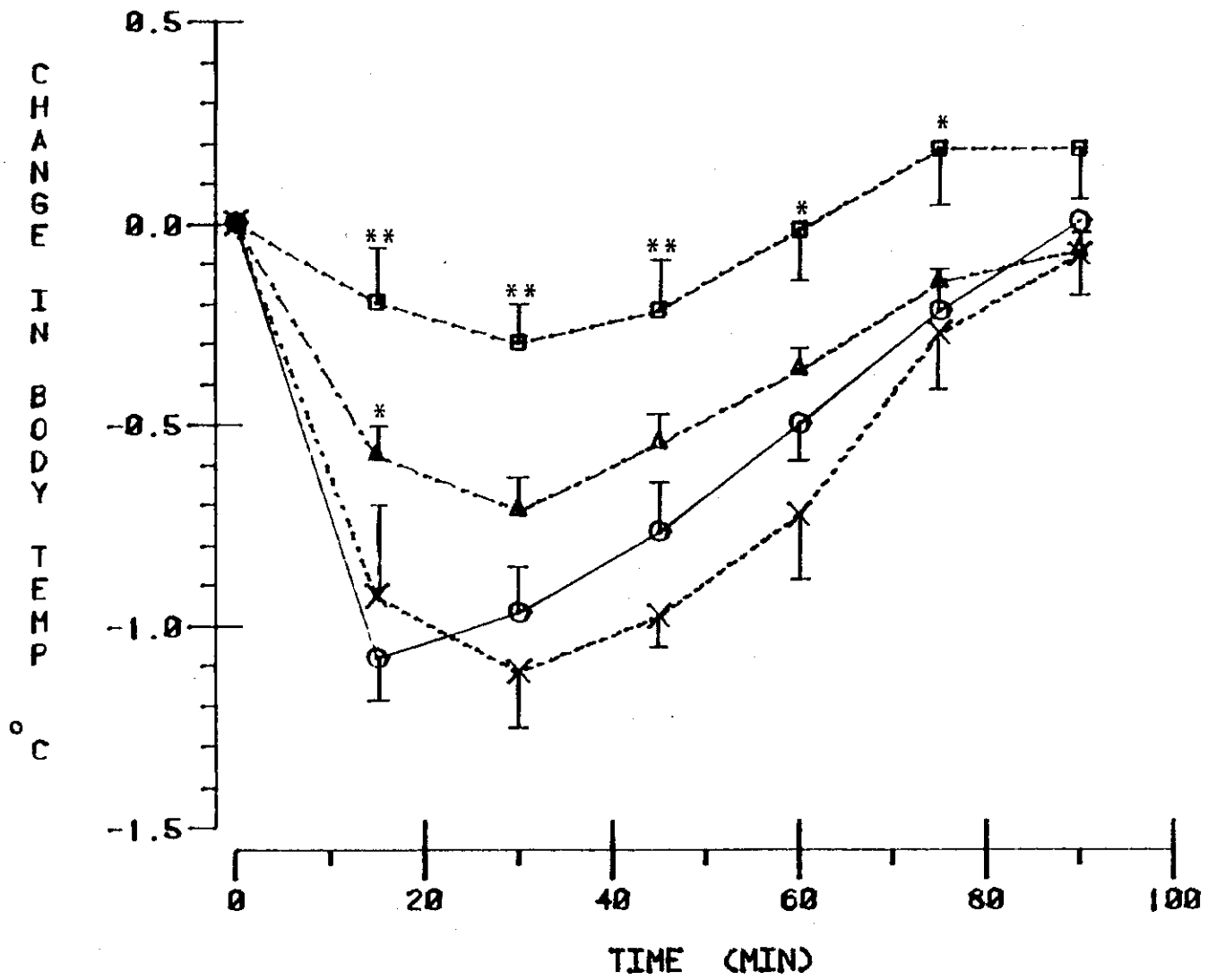
TRZ 1.0/APO 2.0 = Thioridazine HCl 1.0 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

TRZ 1.7/APO 2.0 = Thioridazine HCl 1.7 mg/kg followed by apomorphine HCl 2.0 mg/kg (N=6)

TRZ 3.0/APO 2.0 = Thioridazine HCl 3.0 mg/kg followed by apomorphine Hcl 2.0 mg/kg (N=6)

* = Significantly different rats injected with H20/APO 2.0 ($\underline{P} < 0.05$)

** = Highly significantly different from rats injected with H20/APO 2.0 ($\underline{P} < 0.01$)


O H2O / APO 2.0
X TRZ 1.0 /APO 2.0
Δ TRZ 1.7 /APO 2.0
□ TRZ 3.0 /APO 2.0
CHANGE IN BODY TEMP °C
0.5
0.0
-0.5
-1.0
-1.5
0
20
40
60
80
100
TIME (MIN)
**
**
**
*
*
*

at the 45 and 60-minute time intervals (Figure 21). When tested alone, CPZ 3.0 mg/kg significantly lowered the body temperature of the rat 30 and 45 minutes following intraperitoneal injection (Figure 18). The body temperature dropped to a maximum of $-0.48 \pm 0.18^{\circ}C$ (mean $\pm$ SEM) at the 45-minute time interval. The maximum drop in body temperature induced by APO 2.0 mg/kg was highly significantly antagonized when rats were pretreated with TRZ 3.0 mg/kg and significantly antagonized with a pretreatment dose of 1.7 mg/kg (Figure 22). Pretreatment with TRZ 1.0 mg/kg potentiated the hypothermic action of APO between 30 and 90 minutes following the APO injection to a degree that was not significant (Figure 21). When tested alone, TRZ 3.0 mg/kg did not significantly affect body temperature in the rat (Figure 17).

5. Regression Analysis

The log molar ED_{50} of HAL, LEN, CPZ, and TRZ of one of the four pharmacological parameters measured were compared to each of the other three using regression analysis. In this analysis, the correlation coefficients of body temperature compared to stereotyped behavior, locomotor activity and catalepsy were 0.92, 0.94 and 0.92, respectively, while in the analysis of the other three pairings of pharmacological parameters, all had correlation coefficients of 0.99 (Table X). In this analysis, only the log molar ED_{50} of TRZ on its ability to block APO-induced hypothermia

compared to the log molar ED_{50} of TRZ on its ability to block APO-induced stereotyped behavior and locomotor activity and its ability to induce catalepsy deviates from the regression line when comparing one pharmacological parameter to each of the others (Figures 23, 24, 25, 26, 27, 28).

In order to further examine this observation, TRZ was excluded from the regression analysis and only the log molar ED_{50} values of HAL, LEN and CPZ between one pharmacological parameter and each of the others were compared. The correlation coefficients of body temperature compared to stereotyped behavior, locomotor activity and catalepsy improved to 0.99, 0.98 and 0.99, respectively, (Table XI). The regression lines generated in this analysis further demonstrate the deviation of TRZ from the regression line when body temperature is compared to each of the other pharmacological parameters (Figures 23, 24, 25, 26, 27, 28).

Table VI. The ED_{50} and mole ED_{50} of haloperidol lactate on its ability to block apomorphine hydrochloride-induced effects and to induce catalepsy.

Parameters	ED_{50}	mole ED_{50}
Body Temperature	0.09 (0.07-0.11)	1.94×10^{-7} (1.54×10^{-7} - 2.45×10^{-7})
Stereotyped Behavior	0.06 (0.05-0.07)	1.40×10^{-7} (1.26×10^{-7} - 1.56×10^{-7})
Locomotor Activity	0.05 (0.04-0.06)	1.02×10^{-7} (8.28×10^{-8} - 1.25×10^{-7})
Catalepsy	0.87 (0.57-1.32)	1.87×10^{-6} (1.23×10^{-6} - 2.83×10^{-6})

Values for ED_{50} and mole ED_{50} are reported as mg/kg (95% C.L.) and moles/kg (95% C.L.), respectively.

Table VII. The ED_{50} and mole ED_{50} of lenperone hydrochloride on its ability to block apomorphine hydrochloride-induced effects and to induce catalepsy.

Parameters	ED_{50}	mole ED_{50}
Body Temperature	0.23 (0.17-0.32)	5.74×10^{-7} (4.20×10^{-7} - 7.86×10^{-7})
Stereotyped Behavior	0.14 (0.12-0.17)	3.54×10^{-7} (2.94×10^{-7} - 4.25×10^{-7})
Locomotor Activity	0.66 (0.04-0.09)	1.52×10^{-7} (1.02×10^{-7} - 2.24×10^{-7})
Catalepsy	1.09 (0.98-1.22)	2.68×10^{-6} (2.40×10^{-6} - 3.00×10^{-6})

Values for ED_{50} and mole ED_{50} are reported as mg/kg (95% C.L.) and moles/kg (95% C.L.) respectively.

Table VIII. The ED_{50} and mole ED_{50} of chlorpromazine hydrochloride on its ability to block apomorphine hydrochloride-induced effects and to induce catalepsy.

Parameters	ED_{50}	mole ED_{50}
Body Temperature	2.37 (2.07-2.70)	6.66×10^{-6} (5.83×10^{-6} - 7.61×10^{-6})
Stereotyped Behavior	1.46 (1.43-1.49)	4.11×10^{-6} (4.02×10^{-6} - 4.20×10^{-6})
Locomotor Activity	1.02 (0.81-1.28)	2.86×10^{-6} (2.27×10^{-6} - 3.61×10^{-6})
Catalepsy	6.20 (5.97-6.44)	1.74×10^{-5} (1.68×10^{-5} - 1.81×10^{-5})

Values for ED_{50} and mole ED_{50} are reported as mg/kg (95% C.L.) and moles/kg (95% C.L.), respectively.

Table IX. The ED_{50} and mole ED_{50} of thioridazine hydrochloride on its ability to block apomorphine hydrochloride-induced effects and to induce catalepsy.

Parameters	ED_{50}	mole ED_{50}
Body Temperature	2.17 (1.88-2.50)	5.32×10^{-6} (4.62×10^{-6} - 6.13×10^{-6})
Stereotyped Behavior	11.73 (8.80-15.64)	2.88×10^{-5} (2.16×10^{-5} - 3.84×10^{-5})
Locomotor Activity	4.08 (3.27-5.09)	1.00×10^{-5} (8.20×10^{-6} - 1.28×10^{-5})
Catalepsy	23.28 (22.08-24.53)	5.72×10^{-5} (5.42×10^{-5} - 6.03×10^{-5})

Values for ED_{50} and mole ED_{50} are reported as mg/kg (95% C.L.) and moles/kg (95% C.L.), respectively.

Table X. Regression analysis of the logarithm of the molar ED_{50} of haloperidol lactate, lenperone hydrochloride, chlorpromazine hydrochloride and thioridazine hydrochloride in a series of four pharmacological parameters; body temperature, stereotyped behavior, locomotor activity and catalepsy.

Analysis of:	y-intercept	slope	r
Body Temperature versus Stereotyped behavior	1.74	1.29	0.92
Body Temperature versus Locomotor Activity	1.08	1.22	0.94
Body Temperature versus Catalepsy	-0.03	0.86	0.92
Stereotyped Behavior versus Locomotor Activity	-0.75	0.92	0.99
Stereotyped Behavior versus Catalepsy	-1.21	0.66	0.99
Locomotor Activity versus Catalepsy	-0.71	0.72	0.99

Table XI. Regression analysis of the logarithm of the molar ED_{50} of haloperidol lactate, lenperone hydrochloride and chlorpromazine hydrochloride (excluding thioridazine hydrochloride in this analysis) in a series of four pharmacological parameters; body termperature, stereotyped behavior, locomotor activity and catalepsy.

Analysis of:	y-intercept	slope	r
Body Temperature versus Stereotyped Behavior	-0.41	0.96	0.99
Body Temperature versus Locomotor Activity	-0.48	0.99	0.98
Body Temperature versus Catalepsy	-1.40	0.65	0.99
Stereotyped Behavior versus Locomotor Activity	-0.03	1.03	0.99
Stereotyped Behavior versus Catalepsy	-1.10	0.68	0.99
Locomotor Activity versus Catalepsy	-1.11	0.66	0.99

Figure 23. Regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl, chlorpromazine HCl and thioridazine HCl in stereotyped behavior versus the log molar ED_{50} of the same compounds in body temperature (dashed line). The solid line represents the regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl and chlorpromazine HCl in stereotyped behavior versus the log molar ED_{50} of the same compounds in body temperature. (vertical and horizontal bars indicate 95% confidence limits; for values, see Tables VI through IX; for y-intercept, slope and r values see Tables X and XI).

Key: HAL = Haloperidol lactate

LEN = Lenperone hydrochloride

CPZ = Chlorpromazine hydrochloride

TRZ = Thioridazine hydrochloride

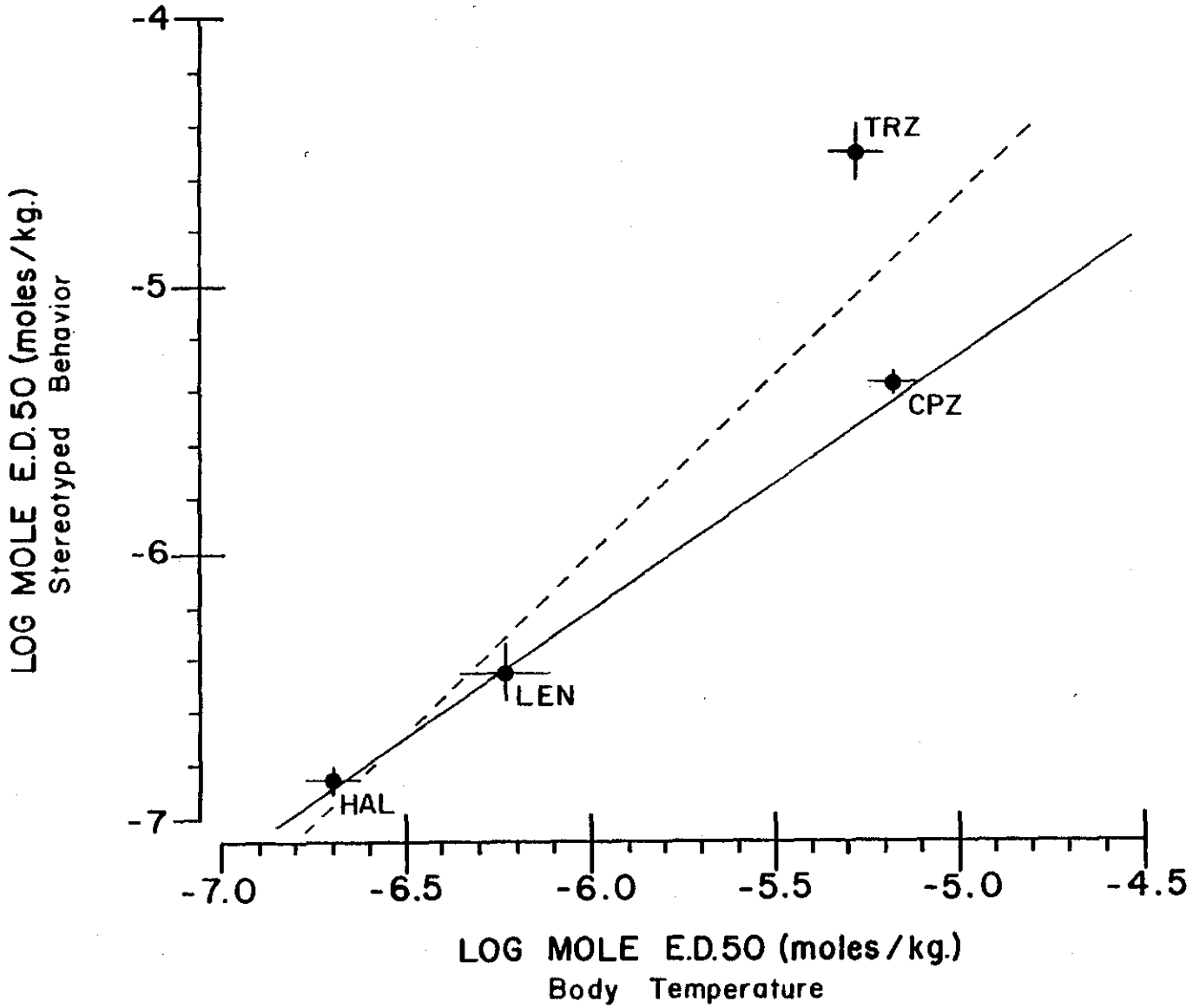

-4
-5
-6
-7
TRZ
CPZ
LEN
HAL
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
LOG MOLE E.D.50 (moles/kg.)
Stereotyped Behavior
LOG MOLE E.D.50 (moles/kg.)
Body Temperature

Figure 24. Regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl, chlorpromazine HCl and thioridazine HCl in locomotor activity versus the log molar ED_{50} of the same compounds in body temperature (dashed line). The solid line represents the regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl and chlorpromazine HCl in locomotor activity versus the log molar ED_{50} of the same compounds in body temperature. (vertical and horizontal bars indicate 95% confidence limits; for values, see Tables VI through IX; for y-intercept, slope and r values, see Tables X and XI).

Key: HAL = Haloperidol lactate

LEN = Lenperone hydrochloride

CPZ = Chlorpromazine hydrochloride

TRZ = Thioridazine hydrochloride

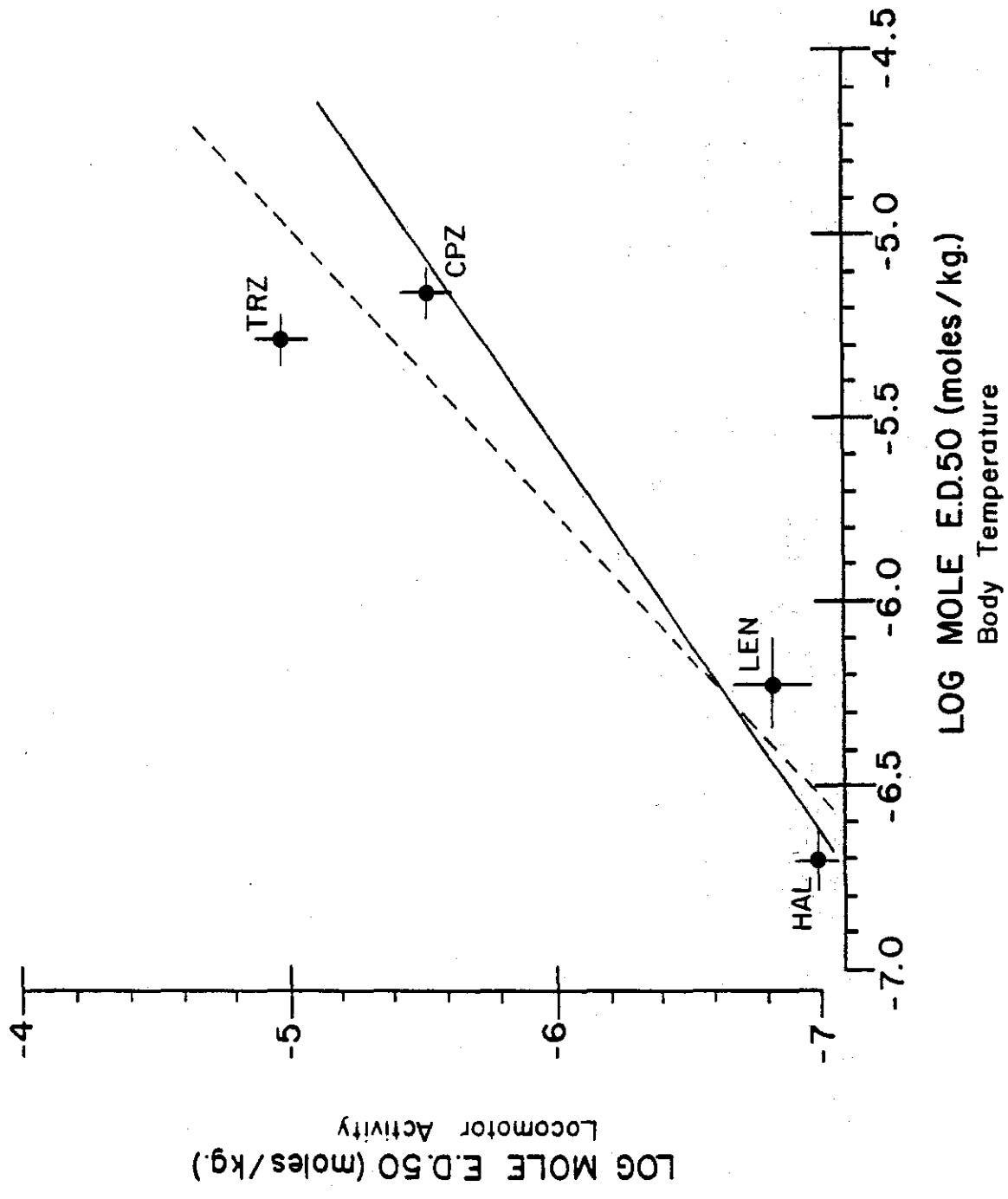

LOG MOLE E.D.50 (moles/kg.)
Locomotor Activity
-4
-5
-6
-7
LOG MOLE E.D.50 (moles/kg.)
Body Temperature
-4.5
-5.0
-5.5
-6.0
-6.5
-7.0
TRZ
CPZ
LEN
HAL

Figure 25. Regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl, chlorpromazine HCl and thioridazine HCl in catalepsy versus the log molar ED_{50} of the same compounds in body temperature (dashed line). The solid line represents the regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl and chlorpromazine HCl in catalepsy versus the log molar ED_{50} of the same compounds in body temperature. (vertical and horizontal bars indicate 95% confidence limits; for values, see Table VI through IX; for y-intercept, slope and r values, see Tables X and XI).

Key: HAL = Haloperidol lactate

LEN = Lenperone hydrochloride

CPZ = Chlorpromizaine hydrochloride

TRZ = Thioridazine hydrochloride

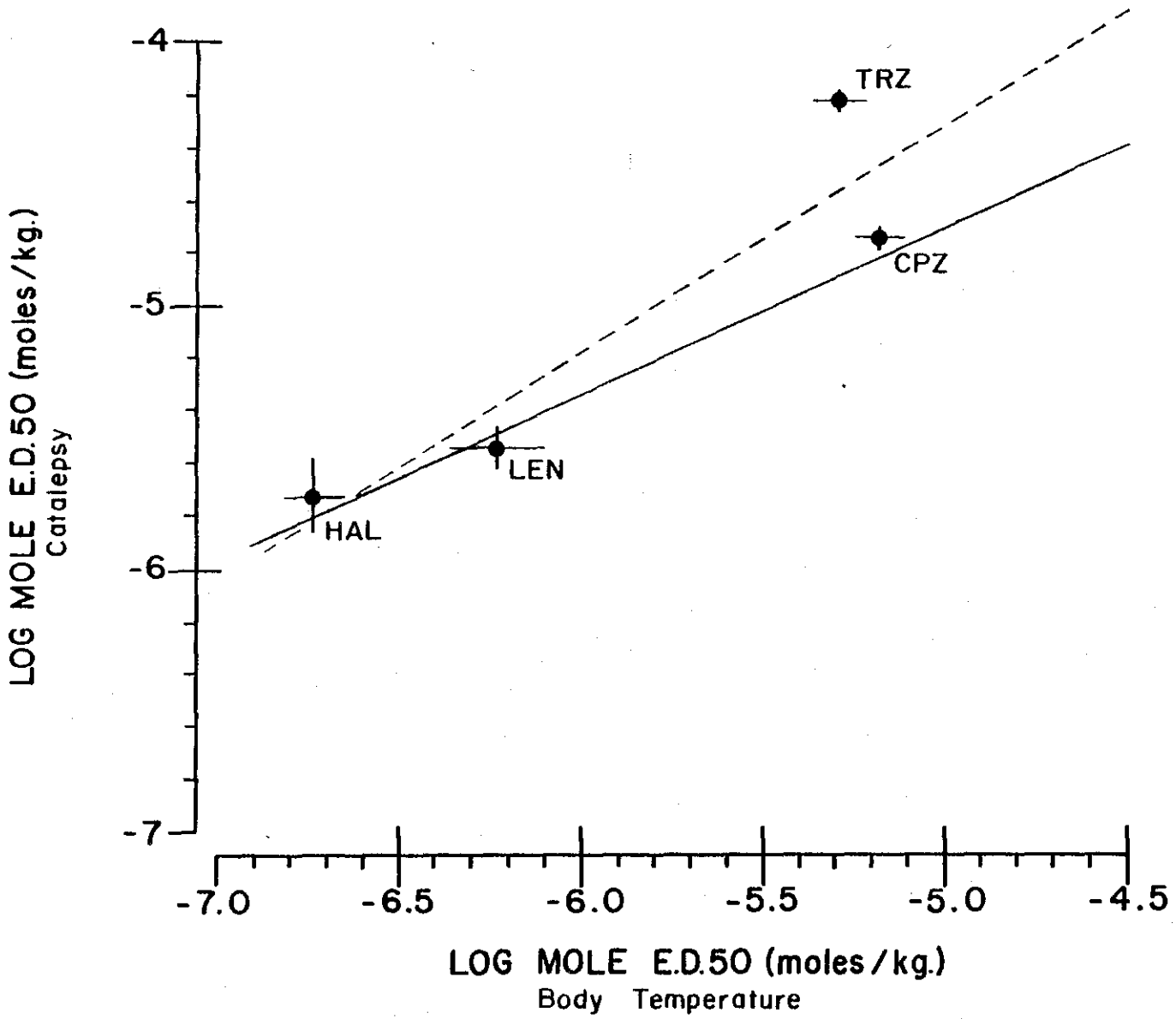

-4
-5
-6
-7
TRZ
CPZ
LEN
HAL
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
LOG MOLE E.D.50 (moles/kg.)
Catalepsy
LOG MOLE E.D.50 (moles/kg.)
Body Temperature

Figure 26. Regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl, chlorpromazine HCl and thioridazine HCl in locomotor activity versus the log molar ED_{50} of the same compounds in stereotyped behavior (dashed line). The solid line represents the regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl and chlorpromazine HCl in locomotor activity versus the log molar ED_{50} of the same compounds in stereotyped behavior. (vertical and horizontal bars indicate 95% confidence limits; for values, see Tables VI through IX; for y-intercept, slope and r values, see Tables X and XI).

Key: HAL = Haloperidol lactate

LEN = Lenperone hydrochloride

CPZ = Chlorpromazine hydrochloride

TRZ = Thioridazine hydrochloride

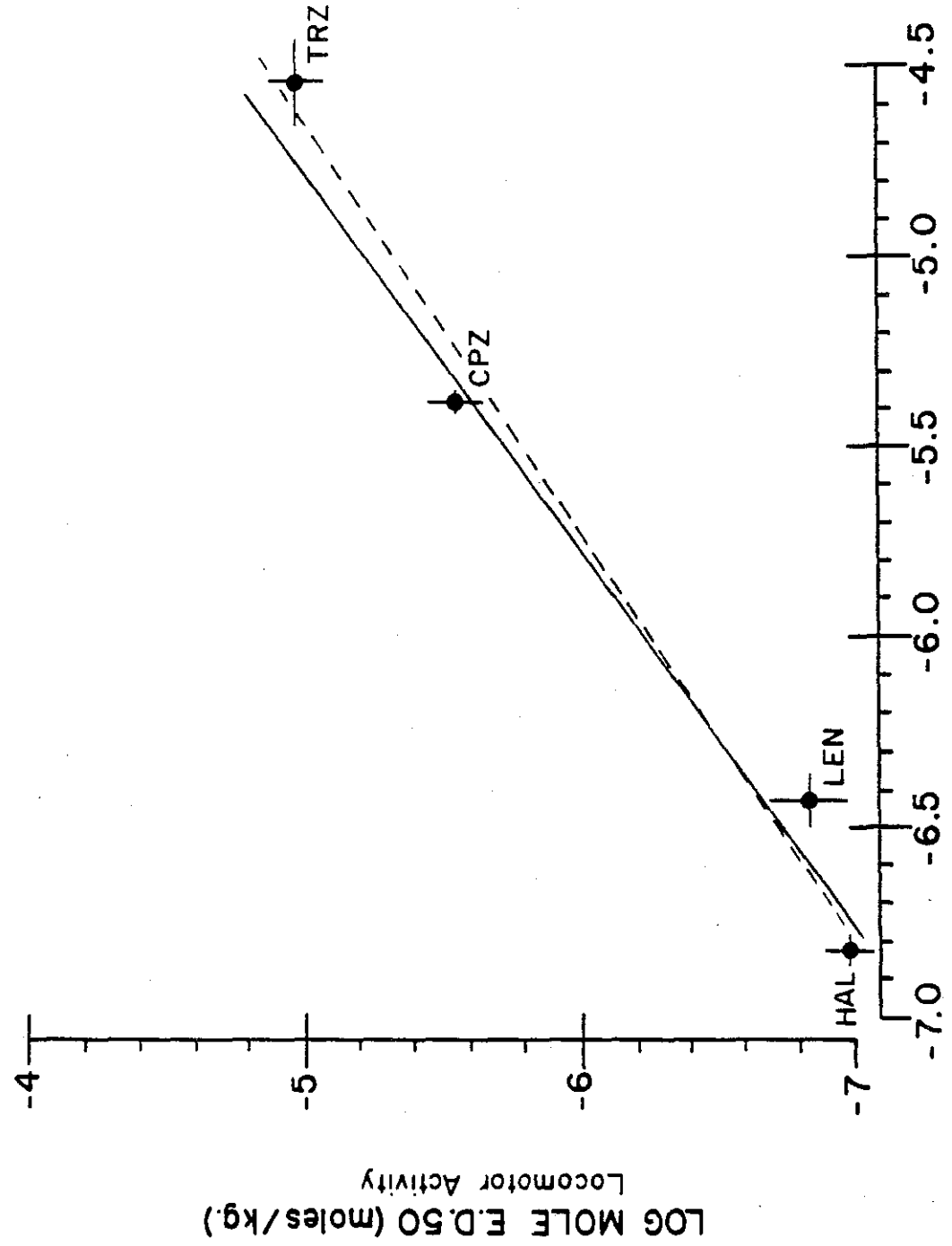

LOG MOLE E.D.50 (moles/kg.)
Locomotor Activity
-4
-5
-6
-7
HAL
LEN
CPZ
TRZ
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
LOG MOLE E.D.50 (moles/kg.)
Stereotyped Behavior

Figure 27. Regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl, chlorpromazine HCl and thioridazine HCl in catalepsy versus the log molar ED_{50} of the same compounds in stereotyped behavior (dashed line). The solid line represents the regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl and chlorpromazine HCl in catalepsy versus the log molar ED_{50} of the same compounds in stereotyped behavior. (vertical and horizontal bars indicate 95% confidence limits; for values, see Table VI through IX; for y-intercept, slope and r values, see Tables X and XI).

Key: HAL = Haloperidol lactate

LEN = Lenperone hydrochlorid

CPZ = Chlorpromazine hydrochloride

TRZ = Thioridazine hydrochloride

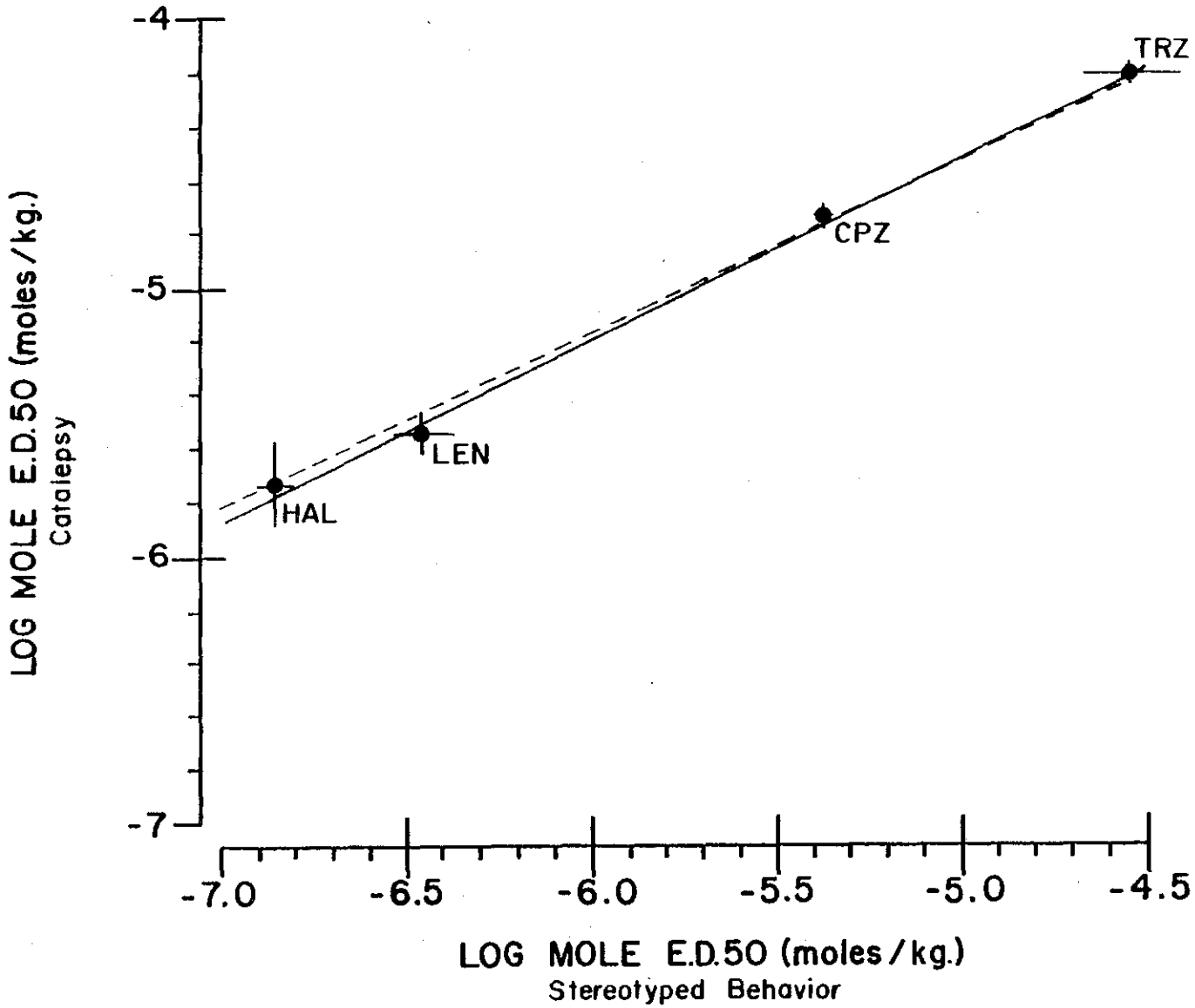

-4
-5
-6
-7
TRZ
CPZ
LEN
HAL
LOG MOLE E.D.50 (moles/kg.)
Catalepsy
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
LOG MOLE E.D.50 (moles/kg.)
Stereotyped Behavior

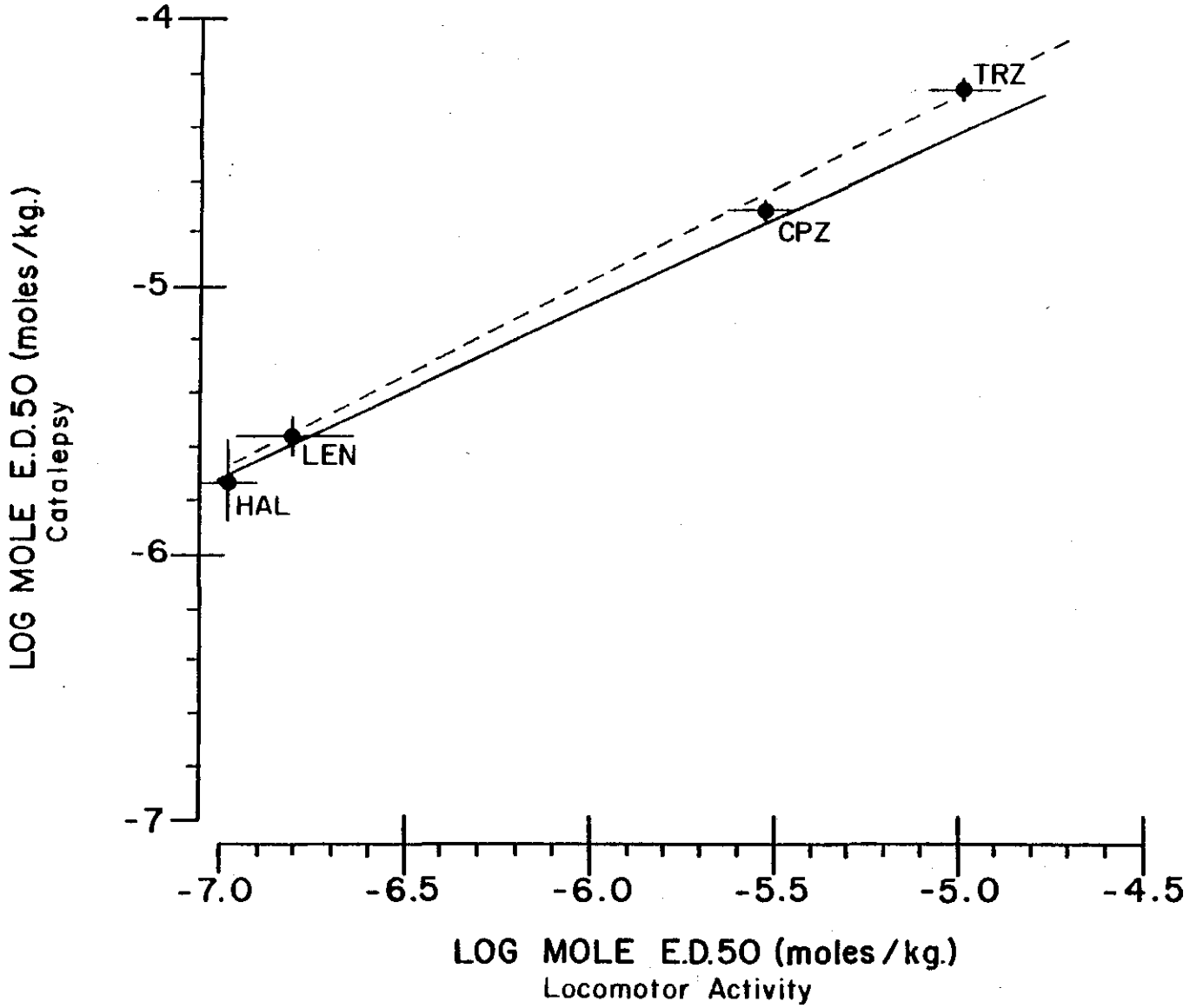
Figure 28. Regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl, chlorpromazine HCl and thioridazine HCl in catalepsy versus the log molar ED_{50} of the same compounds in locomotor activity (dashed line). The solid line represents the regression line of the log molar ED_{50} of haloperidol lactate, lenperone HCl and chlorpromazine HCl in catalepsy versus the log molar ED_{50} of the same compounds in locomotor activity. (vertical and horizontal bars indicate 95% confidence limits; for values, see Table VI through IX; for y-intercept, slope and r values, see Tables X and XI).

Key: HAL = Haloperidol lactate

LEN = Lenperone hydrochloride

CPZ = Chlorpromazine hydrochloride

TRZ = Thioridazine hydrochloride


-4
-5
-6
-7
TRZ
CPZ
LEN
HAL
LOG MOLE E.D.50 (moles/kg.)
Catalepsy
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
LOG MOLE E.D.50 (moles/kg.)
Locomotor Activity

DISCUSSION

The ultimate objective of this research project was to provide further evidence supporting a hypothesis of multiple receptor types for DA in mammalian CNS tissue. The research in this thesis was to investigate the possibilities of gross differences in selectivity for DA receptors by a series of neuroleptic compounds. In the past, few investigators had considered the DA receptor as anything other than a single entity, but investigators have since turned to determining whether there are DA receptor subtypes. A stage has now been reached where there exist numerous subtypes. However, since these have been created from the use of diverse techniques and often times from a compilation of data from different laboratories, the evidence for the existence of multiple receptor types for DA is not always entirely convincing. In this study, all the data was gathered and compiled from standardized procedures in one laboratory and treated by a method of analysis similar to that successfully employed by Portoghese (1965) in investigating analgesic receptors.

In this research project, HAL, LEN, CPZ and TRZ were subjected to a battery of whole animal screening procedures.

These pharmacological tests included antagonism of APO-induced locomotor activity, stereotyped behavior and hypothermia and induction of catalepsy in rats. Systemic ED_{50} values as well as time-activity relationships were determined.

The present study indicates that the observed effects of APO are due to an agonistic effect on dopaminergic mechanisms. There is convincing evidence in the literature correlating the stimulation of central DA receptors to rat locomotor activity (Maj et al., 1972b; Buus Lassen, 1977), stereotyped behavior (Ernst, 1967; Anden et al., 1967) and hypothermia (Kruk, 1972). There is also evidence showing that when DA antagonists are given to rats, catalepsy results (Costall and Naylor, 1974a). The ability of the DA antagonists HAL, LEN, CPZ and TRZ to abolish the effects of APO as well as induce a state of catalepsy in the present experiments is presumptive evidence that APO and the DA antagonists used in this study exert their effects via DA receptors. This is in agreement with other findings in rats that APO induces locomotor stimulation (Di Chiara and Gessa, 1978; Anden et al., 1967; Iversen, 1975), stereotypy (Ernst, 1967; Randrup and Munkvad, 1974) and hypothermia (Barnett et al., 1972; Cox et al., 1978) and that DA antagonists not only block locomotor stimulation, stereotypic and hypothermic effects of APO (Buus Lassen, 1974; 1977; Di Chiara and Gessa, 1978; Fuxe and Sjöqvist,

1972) but also induce a state of catalepsy in the rat (Munkvad et al., 1968).

The neuroleptics selected for this study include compounds reported to possess similar antipsychotic properties with differential incidences of EPS. HAL and CPZ, which are used as antipsychotic agents and are known to cause a high incidence of EPS (Crane, 1967; Hanlon et al., 1965), are referred to as typical neuroleptics. LEN and TRZ, which possess antipsychotic properties and non-typical neuroleptic properties in animal models (Quock and Louie, 1977; Ljungberg and Ungerstedt, 1978), give a low degree of EPS (Harris, 1975; Carranza and Toro, 1974), and are referred to as atypical neuroleptics.

This study shows that the central DA antagonists employed produce common pharmacological actions. They all inhibit APO-induced locomotor activity, stereotyped behavior and hypothermia as well as induce catalepsy. There are, however, differences in potency. The data obtained from this study indicate that in the pharmacological parameters measured, HAL is the most potent followed in order by LEN, CPZ and TRZ. An exception was observed in the body temperature studies where TRZ was slightly more potent than CPZ in blocking APO-induced hypothermia.

TRZ was qualitatively different from HAL, LEN and CPZ in that the APO-induced locomotion was blocked at doses

that did not reduce the APO-induced stereotyped behavior or induce catalepsy (Table II, III, IV). The ED_{50} for TRZ's ability to block APO-induced locomotor activity was sufficient to completely block APO-induced hypothermia (Table IX). LEN, HAL and CPZ were able to block APO-induced locomotor activity at doses that blocked APO-induced stereotyped behavior and hypothermia but did not induce catalepsy (Table II, III, IV, V). The cataleptogenic ED_{50} of all four DA-antagonists is higher than the anti-apomorphine dose (Table VI, VII, VIII, IX).

The results in the present study on the antagonisms of the APO-induced effects agree with previous studies since TRZ has been shown not to antagonize APO-induced stereotyped behavior or induce catalepsy at doses that blocked APO-induced locomotor activity, while HAL and CPZ have been shown to antagonize APO-induced locomotor activity and stereotypy at equipotent doses (Buus Lassen, 1977; Ljungberg and Ungerstedt, 1978; Niemegeers and Janssen, 1979). Although pretreatment with LEN was observed to reduce the thermotropic and locomotor actions of APO in rabbits and rats while leaving the intensity of APO-induced compulsive gnawing unaltered (Quock and Louie, 1977), this study clearly shows that LEN antagonized APO-induced stereotypy behavior and hypothermia at doses that block APO-induced locomotor activity. This also seems to be inconsistent with clinical observations in which LEN was reported to

produce a lower-than-expected, based on neuroleptic potency, incidence of EPS (Harris, 1975).

The determination of systemic ED_{50} values and time-activity relationships for these neuroleptics in the pharmacological parameters previously mentioned will not necessarily show selectivity of the agents for DA receptors in particular regions of the CNS. However, if the activity changes resulting from structurally different neuroleptics were compared to one another by means of regression analysis, gross differences in selectivity for DA receptors could be demonstrated quantitatively. A plot of the log molar ED_{50} of HAL, LEN, CPZ and TRZ in one pharmacological parameter versus the log mole ED_{50} values of these neuroleptics in a second pharmacological parameter should yield a regression line with a high correlation coefficient if the compounds are binding to receptor sites with identical 3-dimensional structure (topography) and chemoselectivity. Conversely, if the DA receptors influencing the different pharmacological parameters are topographically dissimilar, then the plot of the activity in one DA function versus the activity of a second DA function would not yield a linear correlation. This non-parallel change in activity indicates that the drugs being tested are binding to non-identical receptor sites (isoreceptors) in causing their various pharmacological effects. Assuming that isoreceptors do exist, if the compounds being tested have isoreceptor selectivity then the

regression correlation plot showing the activity of a particular series of compounds in a pharmacological parameter governed by a population of DA receptors from one brain region may not produce a linear correlation with the activity of the same series of compounds in a pharmacological parameter governed by a population of DA receptors from either the same or another brain region. By the same rationale, the extent of activity changes in one pharmacological parameter by that same series of compounds would be expected to correlate with those compounds' pharmacological findings believed to be associated with the same population of DA receptors.

According to this mode of analysis, the activity changes reuslting from the different neuroleptics parallel each other for the various parameters measured. The plot of the pharmacological activity in one DA function versus the activity in a second DA function yields a linear correlation in all cases with correlation coefficients ranging from 0.92 to 0.99. The parallel changes evident in the analysis of each pair of biological functions indicate that the compounds are binding to identical receptor sites or are not selective among isoreceptors.

On closer examination of the regression analysis plots, only the log molar ED_{50} of TRZ on its ability to block APO-induced hypothermia compared to the log molar ED_{50} of TRZ on its ability to block APO-induced stereotyped behavior and

locomotor activity and its ability to induce catalepsy deviates from the regression line when comparing the log molar ED_{50} of HAL, LEN and CPZ in one pharmacological parameter to each of the others (Figure 23, 24, 25, 26, 27, 28). As it appears that HAL, LEN, CPZ and TRZ exert their effects via the DA receptor in the pharmacological parameters tested, the peculiarity of TRZ in the regression analysis plots and its ability to block APO-induced hypothermia at doses that do not reduce APO-induced locomotor activity or stereotyped behavior or induce a state of catalepsy may be due to four different possibilities.

First, the metabolism of TRZ may result in a more active metabolite in the area of the hypothalamic thermoregulatory center or a more inactive form in other parts of the brain known to contain dopaminergic neuronal tracts such as the limbic forebrain or the nigrostriatal system. As shown by the studies of Zehnder et al. (1962), the metabolism of TRZ is mainly by demethylation of the nitrogen atom of the side chain to produce northioridazine. The next metabolic step is oxidation of the two sulphur atoms to sulphones and sulphoxides. Northioridazine is therapeutically active and the side-chain sulphone derivative of TRZ is used as a neuroleptic agent (Hopf and Eckert, 1971). As far as hard knowledge of the metabolic fate of neuroleptic drugs is concerned, there is no evidence to support a differential metabolism of TRZ or any of the other

neuroleptics tested in the brain to account for the results obtained with TRZ in this study.

Second, the apparent selectivity of neuroleptic action may result from differential distribution of TRZ in the CNS. The distribution pattern for TRZ was determined by Hopf and Eckert (1971) in the rat brain using radioactive TRZ. Five minutes after i.v. injection, the activity of ^{14}C-thioridazine was found to be particularly high in the limbic system whereas the archicortical hippocampus and the hypothalamus contained little labelled substance. After periods of 1 to 24 hours the activity was situated mainly in the forebrain, the hippocampus, neocortex and thalamus whereas a gradual decline in activity was seen in all other brain regions. Since the hypothalamus, which generally has a very permeable blood-brain barrier, showed low activity, the effect of the barrier cannot be very great. The distribution pattern thus cannot be accounted for in simple terms, and is apparently determined by a number of different factors. Although the pattern of distribution for TRZ reveals a low content in the hypothalamus, one cannot rule out the possibility that this structure may have a high enough concentration of TRZ to evoke a pharmacological response. In order to eliminate drug distribution problems, the neuroleptic agents employed in this study could be administered in microquantities directly into CNS regions where various DA neuronal systems are known to be located. This procedure would localize

the antagonists in the anatomical regions of the brain where DA neuronal functions vary.

Third, a number of studies have been carried out to measure the antimuscarinic potencies of various neuroleptics. Binding studies using specific radiolabelled ligands for muscarinic receptors indicate that TRZ belongs to the class of drugs endowed with potent antimuscarinic properties (Miller and Hiley, 1975; Laduron and Leysen, 1978). Similar results were obtained with *in vivo* studies using mydriatic activity in mice and *in vitro* studies using guinea-pig trachea in which TRZ was rated as a potent antimuscarinic agent, more so than CPZ or HAL (Pearl *et al.*, 1976).

There is considerable evidence to support the concept that there exists a modulatory relationship between cholinergic and dopaminergic neuronal systems in the CNS. The majority of the literature concerning the neuroanatomical basis of the interaction between these two neurotransmitter systems involve the extrapyramidal system (Kelly and Miller, 1975; Muller and Seeman, 1974; Morpurgo, 1962). It has been suggested that the antimuscarinic property of TRZ may be responsible at least in part, for its lack of production of EPS when used clinically (Kelly and Miller, 1975).

In the present study, TRZ's failure to block APO-induced effects and induce catalepsy at doses which blocked APO-induced hypothermia in the rat may be the result of its antimuscarinic properties. In addition to TRZ's

antidopaminergic property, its antimuscarinic action may alleviate the dopaminergic effects of APO in the hypothalamic thermoregulatory center further enhancing TRZ's ability to block APO-induced hypothermia. There is evidence to support the hypothesis that APO-induced hypothermia involves a cholinergic link (Glick and Marsanico, 1974). It was reported that APO produced dose-dependent hypothermic effects in mice which were antagonized by scopolamine. However, Cox and Lee (1977a) were unable to block APO-induced hypothermia with scopolamine at doses which were highly effective in antagonizing oxytremorine-induced hypothermia in rats and mice. Since the exact nature of the interaction between the cholinergic and dopaminergic systems in thermoregulation is as yet unclear, TRZ's antimuscarinic property does not provide the evidence to account for the results obtained with TRZ in this study.

Fourth, there is the possibility of multiple receptors for DA in the CNS of the rat. Since TRZ appears to be more selective for DA recpetors of the hypothalamic thermoregulatory center versus the DA receptors of the corpus striatum or the limbic system, the DA receptors governing body temperature regulation in the hypothalamus are presumed to be topographically and chemoselectively dissimilar to DA receptros in the striatal and limbic systems.

In recent years, the available data indicates the existence of numerous subtypes of DA receptors. The clinical

observations of Klawans (1973) provided some of the first available evidence that supported the existence of two types of DA receptors. The concept of a balance between the DA exitatory and inhibitory mechanisms was developed by Cools and Van Rossum (1976). Their critical review developed into a comprehensive account of two distinct DA receptor types in striatal and mesolimbic brain areas.

In behavioral studies classical and atypical neuroleptics differentially antagonized APO-induced stereotypy and locomotion supporting the hypothesis that there exist different DA receptor types in striatal and mesolimbic areas of the brain (Ljungberg and Ungerstedt, 1978). Differential HVA accumulation in these areas of the brain of rats pretreated with classical or atypical neuroleptics further support this multiple receptor hypothesis (Bartholini, 1976; Uzan et al., 1978).

Various studies present evidence that the presynaptic neuron itself might contain DA autoreceptors (Christiansen and Squires, 1974; Aghajanian, 1977) suggesitng pre- and postsynaptic DA receptor types. Radiolabelled ligands have been used in studies which have employed brain lesion techniques in attempts to locate DA agonist/antagonist binding sites presenting further evidence for the existence of pre- and postsynaptic DA receptors (Nagy et al., 1978; Lee et al., 1978).

The study of drug action on the DA-sensitive adenylate

cyclase system has provided a criteria for designation of multiple categories of DA receptors. The proposed DA receptors linked to, or independent of, the DA-sensitive adenylate cyclase has been referred to as D-1 and D-2 respectively (Kebabian and Calne, 1979). Other studies with kainic acid lesions and ablations of the cerebral cortex have provided further evidence to distinguish at least two types of DA receptors in the neostriatum, those linked to adenylate cyclase and those independent of the enzyme located on axon terminals of corticostriatal fibers (Schwarcz et al., 1978b). More recent data indicate that the bovine anterior pituitary contains two distinct DA receptors, one which is not associated with adenylate cyclase and which controls prolactin release, the other adenylate cyclase linked and regulated by GTP with, as of yet, unknown physiological function (Sibley and Creese, 1979; Caron et al., 1978).

In view of the present findings, it appears that TRZ may be blocking a subclass of DA receptors present in the preoptic anterior hypothalamus which are topographically and chemoselectively dissimilar to those DA receptors governing stereotyped behavior, locomotor stimulation and the induction of catalepsy in rats.

To further elucidate this possibility, it would be of interest to determine whether or not other atypical neuroleptics such as clozapine and sulpiride might share TRZ's

differential action upon preoptic anterior hypothalamic DA receptors. To determine whether selective antagonism of these receptors is a result of actual differences in DA receptor types and not simply differential distribution within the CNS, central microinjection techniques would be used in further studies.

Expanding the number of pharmacological tests to include measuring HVA accumulation in the corpus striatum, hypothalamus and limbic forebrain following the administration of neuroleptic drugs may show differential turnover of DA. For example, neuroleptics that produce a selective blockade of DA receptor types found predominantly in the hypothalamus will hence enhance hypothalamic HVA levels with little effect elsewhere in the brain.

The establishment of a DA receptor binding procedure would allow for the direct assessment of the extent of receptor binding of the neuroleptic agents employed in this study in tissues isolated from the striatum, hypothalamus and limbic forebrain. The data from these binding studies as well as the turnover studies would be subjected to regression analysis along with our pharmacological findings.

Finally, it would be most useful to expand this study to incorporate data from clinical studies using the same neuroleptic agents used in the pharmacological, biochemical and binding studies. A clinical profile of a neuroleptic

with respect to its antipsychotic potency and incidence of EPS would indeed make for a more complete study when this data is treated along with the other data using regression analysis.

CONCLUSIONS

The present study in male Wistar rats was designed to support the hypothesis of multiple receptor types for DA in mammalian CNS tissue. Four neuroleptic compounds were subjected to a series of whole animal screening procedures in an effort to demonstrate gross differences in selectivity for DA receptors.

The results of the present study indicate that APO-induced locomotor activity, stereotyped behavior and hypothermia are produced in rats through central stimulation of DA receptors. The ability of the neuroleptics (HAL, LEN, CPZ and TRZ) to abolish the effects of APO as well as induce a state of catalepsy in these experiments is evidence that APO and these DA antagonists exert their effect via DA receptors.

This study shows that LEN was able to antagonize APO-induced stereotyped behavior and hypothermia at doses that blocked APO-induced locomotor activity. This finding is inconsistent with clinical observations in which LEN was reported to produce lower-than-expected EPS (Harris, 1975).

The results of this study were analyzed by the method of regression analysis and revealed parallel activity changes

from HAL, LEN, CPZ and TRZ for each of the four pharmacological parameters measured. Only the log molar ED_{50} of TRZ on its ability to block APO-induced hypothermia compared to the log mole ED_{50} values of TRZ on its ability to block APO-induced stereotyped behavior and locomotor activity and its ability to induce catalepsy deviates from the regression line when comparing the log mole ED_{50} values of HAL, LEN and CPZ in one pharmacological parameter to each of the others.

It may be concluded from this investigation that (i) differential metabolism and/or (ii) differential distribution of TRZ may be responsible for its differential activity at the hypothalamic thermoregulatory center; (iii) that TRZ's antimuscarinic action was responsible for enhancing its ability to block APO-induced hypothermia; and (iv) TRZ may be more selective for a population of DA receptors present in the preoptic anterior hypothalamus which are topographically dissimilar to DA receptors elsewhere in the CNS.

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